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EXPERIMENTAL INFECTIOUS ANGIITIS *

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The possibility that bacterial infection may play a rôle in the pathogenesis of the more chronic forms of vascular disease has long been a subject of discussion. Opinions differ, some reviewers believing that bacteria are of major significance in the etiology of arteriosclerosis (Ophüls,[1] Klotz [2]), while others feel that they are of negligible importance (MacCallum [3]). Statistical and experimental evidence is conflicting. Interest in the subject is kept alive, however, by recent reports such as those of Boyd,[4] who described "acute inflammation" of atheromatous plaques, of Jones and Rogers,[5] who demonstrated diplococci in the walls of coronary arteries, of Benson, Smith and Semenov,[6] who cultured streptococci from coronary arteries at autopsy and used them to produce arterial disease in rabbits, and of Fish, Hand and Keim,[7] who demonstrated bacilli in a calcified aortic plaque in a case of endocarditis due to *Bacillus pyocyaneus*.

It has recently been shown [8] that the artery wall may be exceedingly vascular, especially when thickened, and that such vascularity provides an obvious potential pathway for microorganisms. In order to observe such passage of infection through the artery wall, and also its extension from an adjacent vein, the following experiments were devised.†

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† The first few experiments were carried out by Dr. Alfred E. King.

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Methods

A total of 15 goats was used. This species was selected because it has vessels of a size and structure comparable to those of man. The experimental procedure in the majority of cases was as follows: Under aseptic technic the carotid artery, jugular vein or femoral vessels were exposed. A suspension of bacteria was then injected through a fine hypodermic needle into the outer coat of one of the vessels. An attempt was made to raise a small bleb in the adventitia. In a few cases an infected thread was passed into the vein or the organisms were injected into the lumen of a doubly ligated stretch of vessel. After injection of the organisms the muscles, fascia and skin were closed, care being taken to avoid as far as possible infection of these tissues. The animals were then sacrificed at intervals varying from 24 hours to nearly 3 months.

The organisms used were: (1) *Staphylococcus aureus*; (2) *Bacillus pyocyaneus*, a strain obtained at autopsy from a case in which there were extensive acute lesions of the small vessels of the intestines (*cf.* Fraenkel [9, 10]); (3) *Streptococcus viridans*, a strain which was obtained from an infected tooth and which had been used previously to produce endocarditis in rabbits; and (4) Actinomyces, accidentally, in 1 animal. In the case of the first three, a rather concentrated saline suspension of organisms from a 24 hour broth culture was used.

When the animals were autopsied the vessels at the operative sites were removed, together with a good deal of the surrounding tissue, and fixed *en bloc* in 10 per cent formalin. Multiple sections were then made at intervals of a millimeter or so, and the blocks were studied with a binocular dissecting microscope. Paraffin sections were then made and stained routinely with the Masson trichrome method (Foot's modification [11]) or a combined Weigert's elastic tissue and Masson's trichrome stain. Other stains used occasionally were hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin and the Brown-Brenn stain for bacteria.[12]

Results

Lesions were produced in either artery or vein, or both together, ranging from acute suppurative and proliferative reactions with

or without thrombosis to dense, fibrous intimal plaques. The nature and extent of these lesions varied chiefly with the virulence of the organism and the duration of the experiment. The results are perhaps most conveniently grouped according to the organism used:

(1) *Staphylococcus Aureus:* This organism, which was used in 5 animals, produced in most cases an acute suppurative reaction in the region where it was injected. For instance, when placed in the wall of a vein it usually gave rise to well marked necrosis of the vein wall with an extensive exudate of polymorphonuclear leukocytes and, frequently, a thrombus in the lumen. Of greatest interest was the effect sometimes observed in the neighboring artery. This consisted of an acute arteritis with or without intimal proliferation. In some cases the pathway of extension from vein to artery seemed to be definitely by way of the vasa vasorum. Figure 1 represents the femoral vessels of goat No. 1. In this case a thread soaked in a suspension of staphylococci was passed into the wall of the vein and the animal was sacrificed 4 days later. The vein contains a thrombus and its wall is partially destroyed on one side by a severe suppurative reaction. The tissue intervening between the vein and the artery is edematous but contains only a few leukocytes. In the media of the artery, as shown in the high power drawing, there are collections of polymorphonuclear leukocytes in spaces which apparently are vessels. The intima shows no demonstrable change. The bacterial stain revealed a few cocci in the adventitia of the artery but none in the media or intima.

A somewhat more extensive involvement of the artery is shown in Figure 2. This is a different part of the same vessel illustrated in Figure 1. Here there is a more abundant exudate and hemorrhage in the adventitia of the vessel on the side next the vein. The media shows comparatively little change other than a few polymorphonuclear leukocytes which may lie in the vasa vasorum. The intima, however, shows a rather striking reaction; it is thickened by several layers of cells which overlie a dense layer of fibrin. As will be noted below, such a layer of fibrin has been described in rheumatic lesions of the peripheral arteries.

The extraordinarily rapid intimal proliferation which was sometimes encountered is well illustrated in Figure 6. In this case

the suspension of staphylococci was injected into the adventitia of the carotid artery. With organisms of lower virulence, as will be seen below, the artery wall seemed quite resistant to infection. In this instance, however, after 4 days the reaction was intense and suppurative, almost destroying part of the wall of the artery and extending to the vein, where a thrombus was produced. Figure 6 shows a portion of the intima and a small bit of the media. The intima is thickened by large, actively proliferating cells, probably fibroblasts derived from the scanty subendothelial fibrous layer which is normally found in this vessel. Two mitotic figures are seen in the field. Also, there is seen again a layer of fibrin next to the internal elastic lamella. The media is heavily infiltrated with polymorphonuclear leukocytes (not shown) and is partially necrotic. In the bacterial stain single cocci and diplococci are found occasionally in the media and rarely in the proliferated intima.

(2) *Bacillus Pyocyaneus:* This mildly pathogenic, pyogenic organism appears to have a predilection for blood vessels (Fraenkel,[9, 10] Fish, Hand and Keim[7]). It was found useful because it produced a chronic suppurative reaction without the rapid extension and widespread tissue destruction associated with the staphylococcus. In the 2 goats in which it was used it was injected into the adventitia of the carotid arteries and femoral veins. Only once was an arterial lesion found; this was in a carotid artery, into the wall of which the organisms had been injected 1 week before, and consisted of a severe necrosis of the vessel wall on one side with a mural thrombus and a proliferative reaction in the intima. The femoral veins, however, showed lesions quite constantly. In 1 animal a necrotizing thrombophlebitis was found. In the other, the lesion shown in Figure 3 was encountered. Here the vein wall is involved, 2 weeks after injection of the organisms, by the suppurative change, and the intima shows a remarkably extensive proliferative process composed of cells which again are not readily identifiable. This proliferating mass is vascularized by tiny capillaries, four of which may be seen in the high power drawing. (Note: the erythrocytes of the goat are considerably smaller than those of man.)

(3) *Streptococcus Viridans:* This organism was used in 6 animals with the object of exciting a very mild reaction and of obtaining, if possible, a chronic or healed lesion. No arterial

lesions were found with this organism but interesting venous lesions were encountered.

Two of the animals were used for acute experiments and were sacrificed after 24 and 72 hours respectively. In the former animal the organisms were injected into the walls of the jugular and femoral veins; in the latter they were placed in the lumen of a doubly ligated stretch of the jugular vein and into the wall of the femoral vein. In both animals a very early acute phlebitis was found. This varied from a scattering of polymorphonuclear leukocytes in the adventitial tissues, scarcely more than one would expect from the trauma of operation, to a slightly more severe reaction, with minimal necrosis of fixed tissue and a leukocytic exudate which involved the vein wall to some extent. The evidence of damage to the vessel wall was negligible and consisted in a slight looseness of the tissue, suggesting edema, and swelling of the endothelium of the vasa vasorum of the adventitia and outer media.

In four instances in which the duration of the experiment was 2 weeks (2 animals), 4 weeks and 6 weeks, dense fibrous intimal plaques were found in veins. One of these is illustrated in Figure 4. The intimal thickening is composed of dense collagenous tissue which is well vascularized, particularly at its base. At one point below the plaque there is a slight break in the continuity of the elastic tissue of the media. There is no trace of recent necrosis or of cellular exudate. The lesion was found 6 weeks after injection of *Streptococcus viridans* and may reasonably be regarded as healed. No bacteria were seen in the Brown-Brenn stain.

In several of the vessels into which this organism was injected no reaction whatever could be detected, aside from a possible slight increase in the adventitial connective tissue. It seems probable that the lesion was so mild and evanescent, consisting perhaps only of a little hyperemia and exudation, that resolution occurred and no trace was left after several weeks.

(4) *Actinomyces:* In 1 animal, injected with *B. pyocyaneus*, minute actinomycotic abscesses were found microscopically at two of the injection sites (left carotid artery and left femoral vein) when the animal was sacrificed 6 weeks after operation. Since the lesions are at the sites of injection it seems likely that the organisms were introduced at the time of operation.

The lesion in the carotid artery is shown in Figure 5. In the low power sketch four miliary actinomycotic abscesses can be seen in the adventitia of the artery. It is also evident that the media has practically disappeared on the same side of the vessel and that adjacent to this region there is a massive thickening of the intima. The enlargement at the right shows the "fading out" of the media, the dense collagenous nature of the intimal plaque and one of the abscesses. The high power drawing A illustrates the characteristic colonies of the organism; B shows a small adventitial vessel with a tremendously thickened wall; and in C is seen the margin of the intimal plaque showing its dense yet cellular nature. In this instance the suppurative process is a slow, well circumscribed one and the attendant proliferative response is marked.

In the femoral vein the actinomycotic lesion was similar to the one illustrated in Figure 4.

Discussion

The literature concerning experimental infectious lesions of blood vessels has been summarized by Ophüls [1] and more recently by Klotz.[2] Several points of interest may be derived from such a survey: (1) The animal used was, in the great majority of cases, the rabbit. (2) The organism used was, in most instances, a staphylococcus or a streptococcus, although *B. coli*, *B. typhosus*, and diphtheria bacilli or their toxin are frequently mentioned. (3) The route of injection was usually intravenous or intraperitoneal and gave best results when frequently repeated over a long period of time. (4) The common and characteristic lesion was an intimal nodule or plaque having a hyaline or mucoid fibrous structure; damage to the media and cellular infiltration about the adventitial vasa vasorum are described only occasionally.

The constant use of the rabbit for the experimental study of vascular disease is probably unfortunate. The situation is well stated by Klotz [2] as follows: "The rabbit has its very definite limitations as an experimental animal for the study of human diseases. Its aorta is in structure different from the human vessel and possesses a poor vascular bed in its adventitia. It is not possible to obtain similar results in the vessels of the rabbit, where the mechanism of the nutrition of the aortic wall must be different

from that of the thicker and bulkier structures of larger animals. Nutrition of the entire thickness of the rabbit's aorta can probably be gained by imbibition from the lumen, while this is not the case in the human. These anatomic differences play a very important part in the pathological process which appears in each variety of artery." It is also noteworthy that of the few investigators who have used dogs or monkeys and who have given injections by the intravenous route, several report questionable or entirely negative results (Adler,[13] Clawson,[14] and Benson *et al.*[6]).

Most investigators who have used the intravenous or intraperitoneal route have found it necessary to inject organisms repeatedly over a period of weeks or months before lesions appeared. Hence the earliest stages of the lesions usually have not been described. Also, it has not been clear in most cases whether the lesions were due to actual local invasion of the organisms themselves or to the long continued presence of their toxins in the animal's blood stream.

The method used in the present investigations is admittedly open to criticism in that it introduces artificial conditions associated with the trauma of operation. The traumatic factor can be fairly well controlled, however, by carrying out the full operative procedure and by injecting sterile saline solution instead of bacteria. This was done in 2 animals and a negligible reaction ensued in the adventitial tissues. The great advantage of the method is that the site and time of localization of the organisms are known and the reaction of the tissues can be followed through its various stages.

Only a few workers have used this method. Talke [15] placed a fragment of an agar culture of staphylococci next to the jugular and the femoral veins in dogs, cats and rabbits. The duration of his experiments was from 9 to 74 hours, *i.e.* not long enough for any considerable intimal thickening to develop. Thrombi usually occurred and spaces ("Spalten") in the media contained leukocytes and bacteria.

Sumikawa [16] carried out a series of experiments in which he painted the adventitia of rabbits' arteries with various injurious agents such as silver nitrate, oil of turpentine, and cultures of staphylococci and streptococci. He obtained intimal thickenings associated with inflammation of the media and the adventitia, and

noted that in some instances, in the late stages, the intimal thickening remained without demonstrable residua in the media or adventitia. His animals were allowed to live up to 50 days.

Klotz,[17] in attempting to reproduce the lesions of periarteritis nodosa, introduced various strains of streptococci, isolated from cases of the disease, into the walls of vessels in rabbits. He obtained some cellular infiltration of the adventitia and shows one picture of "mesarteritis and endarteritis" without appreciable intimal thickening. It is interesting to note that he comments on the difficulty of controlling the dose and virulence of the organism.

Buerger,[18] in an attempt at experimental reproduction of the lesions of thromboangiitis obliterans, placed clots from cases of acute migrating phlebitis next to doubly ligated veins in healthy persons. There resulted in 2 cases a thrombophlebitis with foci of miliary giant cells which he regarded as typical of the disease which bears his name.

One of the most interesting aspects of the present experiments was the occasional extension of the infectious process from vein to artery, as illustrated in Figures 1 and 2. In Figure 1, although the vein contains a suppurative thrombus, the tissue lying between it and the artery is fairly intact, although edematous and infiltrated by a few cells. Still, in the wall of the artery, as shown in the high power drawing, there are spaces which contain polymorphonuclear leukocytes and, rarely, a red blood cell. These spaces are not always identifiable as blood vessels, but certainly some of them are. They doubtless correspond to the "Spalten" so well described by Köster,[19, 20] referred to by Talke[15] and others, and considered variously to be blood vessels, lymphatics or tissue spaces. In Figure 2 there are similar, less well defined spaces in the media. Here there is a suppurative hemorrhagic process adjacent to the artery wall, but extension through the media again may be taking place by way of the vasa vasorum.

It seemed of interest to determine whether a direct vascular pathway from vein to artery could be demonstrated by other methods. Therefore, a number of injections were carried out on the vessels of both goat and man. Figure 7 represents a block a few mm. thick of the femoral vessels of a goat, cleared by the Spalteholz method.[21] A segment of the vessels was excised at autopsy with a good deal of the surrounding muscle and connec-

tive tissue. One of the open ends of the vein was then tied and an injection of dilute India ink made through a cannula tied into the other end. The artery was not injected directly. In one part of the cleared specimen the condition illustrated was found. A small tributary of the vein is fed by a plexus of small vessels branching in the adventitia and outer media of the artery. Obviously a retrograde injection of vasa vasorum of the artery from the lumen of the vein has occurred.

Successful injections were not obtained on all attempts, by any means, but were frequent enough to justify the conclusion that there is a fairly free communication between the vasa vasorum of the femoral artery and vein, a fact which was not unsuspected previously (Winternitz, *et al.*[8]). In Figure 8 there is reproduced a drawing of a cleared block of human femoral vessels which were handled in the same way as the goat vessels pictured in Figure 7. There is a fairly extensive injection of the wall of both vein and artery.

These injections demonstrate at least a potential pathway for infection from vein to artery. Such a pathway may have been followed in some of the experiments described in this paper and may be followed not infrequently in human disease. Occasionally in autopsy material one encounters examples of apparent close association between disease of an artery and its concomitant vein. The outstanding example, of course, is thromboangiitis obliterans, where such intimate association is the rule.[22] But instances in cases of angiitis or sclerosis of the peripheral vessels are not uncommon. Several papers, most of them French, have appeared recently on this subject.[23–27] Many of them are clinical reports dealing particularly with the occurrence of arterial spasm in association with phlebitis. A fairly extensive discussion is given by Cornil, Mosinger and Audier,[24] who emphasize the great importance of involvement of the adventitia in phlebitis or arteritis. Through this tissue propagation may occur either "transversely" or "longitudinally," therefore an arteritis need not be exactly opposite a phlebitis of an adjacent vein. They do not stress the vascular pathway but rather point out the importance of involvement of the adventitial nerves in leading to arterial spasm.

The pathogenesis of the intimal proliferation (Figs. 2–6) is interesting but obscure. In cases where the media of the vessel is

partially destroyed, the intimal change may be labeled as the so-called compensatory hyperplasia which may occur from a variety of agents which damage the media, including trauma and simple ligation.[28, 29, 30] In some cases, however, the media shows only slight alteration (Figs. 2, 4 and 6). The nature and origin of the actively proliferating cells of the early lesion (Figs. 2, 3 and 6) are obscure. It is impossible to tell whether or not they arise from endothelium.[31, 32, 33] It seems more likely that they are fibroblasts derived from the subendothelial connective tissue, for they appear to form the dense collagenous plaques of the later lesions (Figs. 4 and 5). Proliferation of new capillaries accompanies them (Figs. 3 and 4). The layer of fibrin sometimes encountered beneath the rapidly proliferating intimal cells (Figs. 2 and 6) is interesting, since it resembles a similar condition in the peripheral arteries in rheumatic fever (VonGlahn and Pappenheimer [34]).

Finally, the relative resistance of the artery wall to direct infection is of some note. As mentioned above, in several cases in which *Streptococcus viridans* and *Bacillus pyocyaneus* were injected directly into the adventitia of the artery, no lesion could be detected either grossly or microscopically. The veins, however, showed some reaction in almost all cases. Whether this difference in susceptibility is associated with the greater vascularity of the vein wall [8] is not clear.

Summary

Various bacteria were introduced under aseptic technic into the adventitial tissue (more rarely on a thread into the lumen or into a doubly ligated stretch) of femoral, jugular and carotid vessels of goats. By varying the virulence of the organism and the duration of the experiment, lesions of both artery and vein were obtained, ranging from acute suppurative and proliferative reactions with thrombosis to old, fibrous intimal plaques. A potential vascular pathway for passage of infection from vein to artery has been demonstrated in both goat and man by injection methods.

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DESCRIPTION OF PLATES

Plate 1

Fig. 1. Femoral vessels of goat No. 1, 4 days after a thread soaked in a suspension of staphylococci was passed into the wall of the vein. Suppurative thrombophlebitis with extension to the adjacent artery.

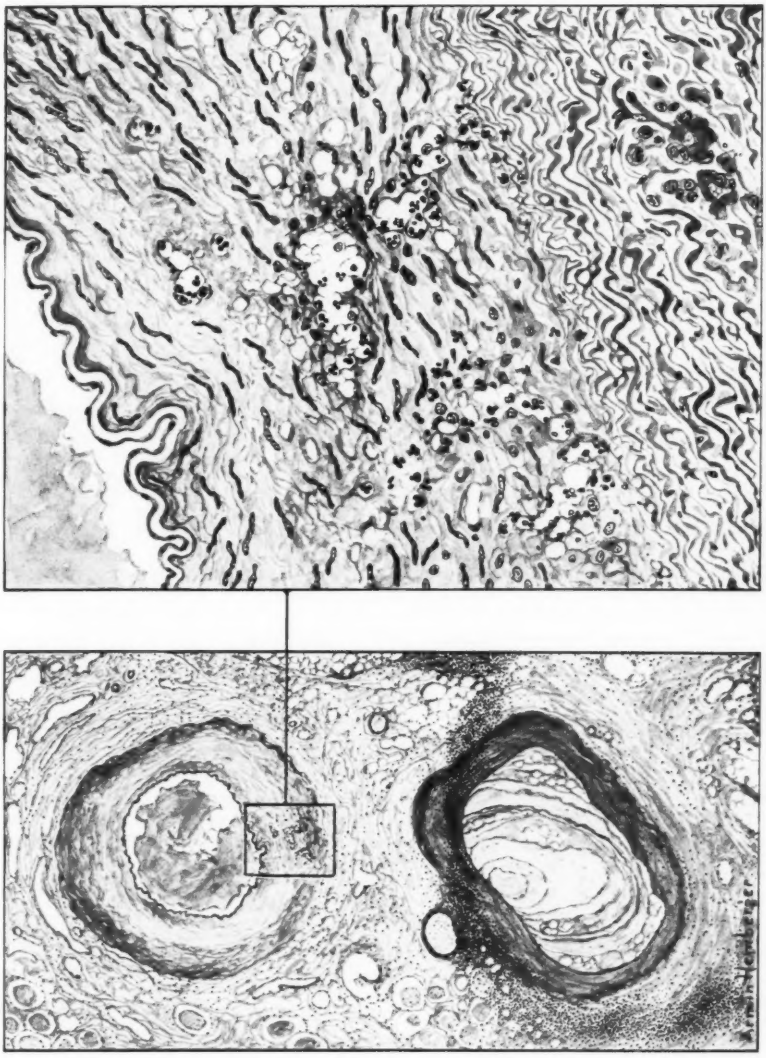
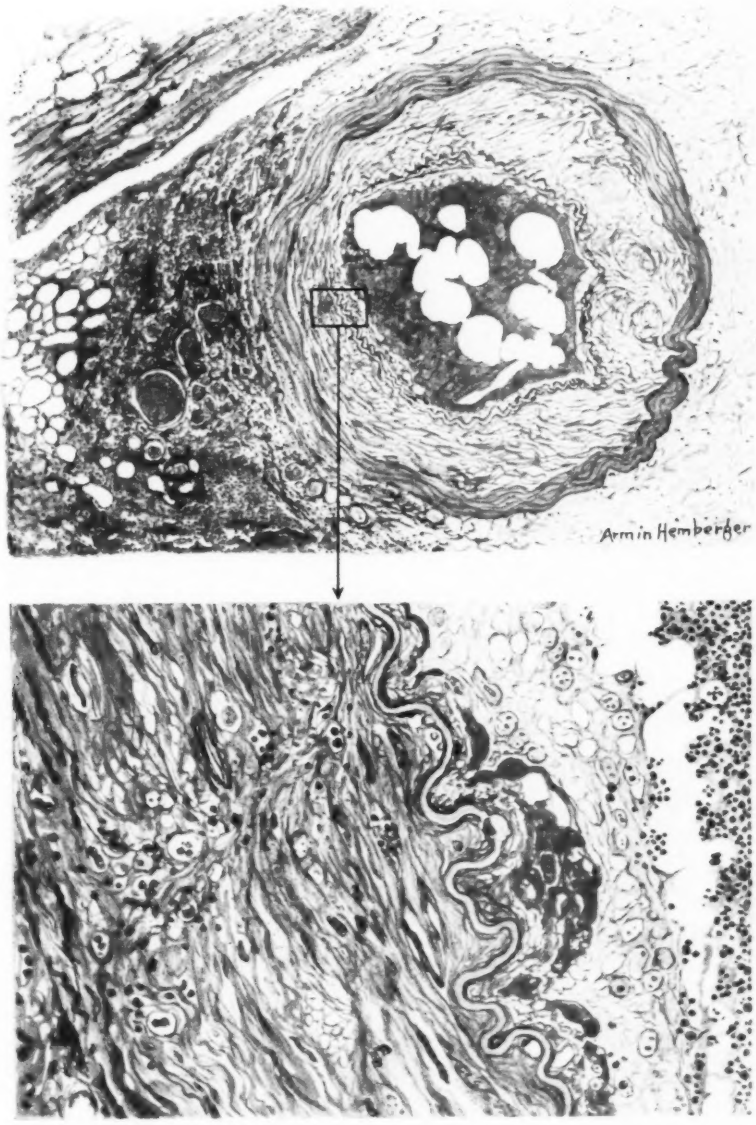


PLATE 2

FIG. 2. Another part of the femoral artery illustrated in Figure 1 and stained by Masson's trichrome method. Leukocytes are present in the wall with intimal proliferation and an overlying mass of fibrin.

FIG. 3. Femoral vein of goat No. 12, 2 weeks after injection of *Bacillus pyocyaneus* into the wall. Suppurative and proliferative phlebitis are present. Weigert's elastic tissue and Masson's trichrome stain.



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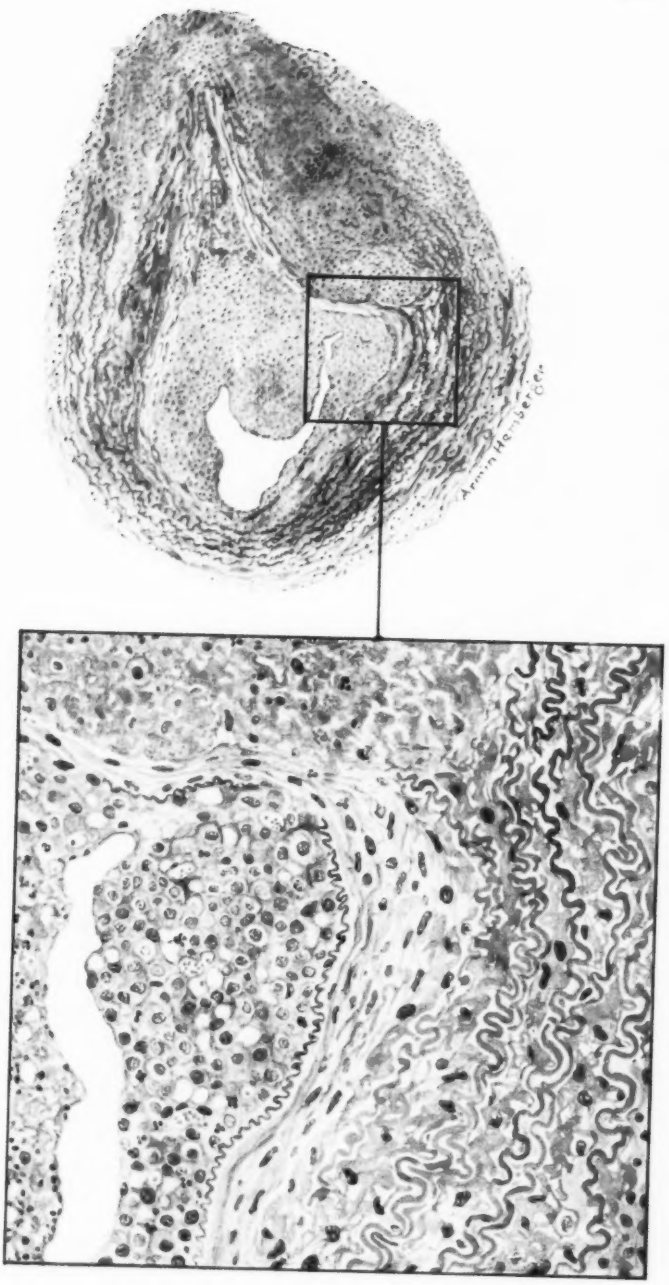

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Experimental Infectious Angiitis

PLATE 3

FIG. 4. Femoral vein of goat No. 7, 6 weeks after injection of *Streptococcus viridans* into the wall. A dense, fibrous intimal plaque remains.

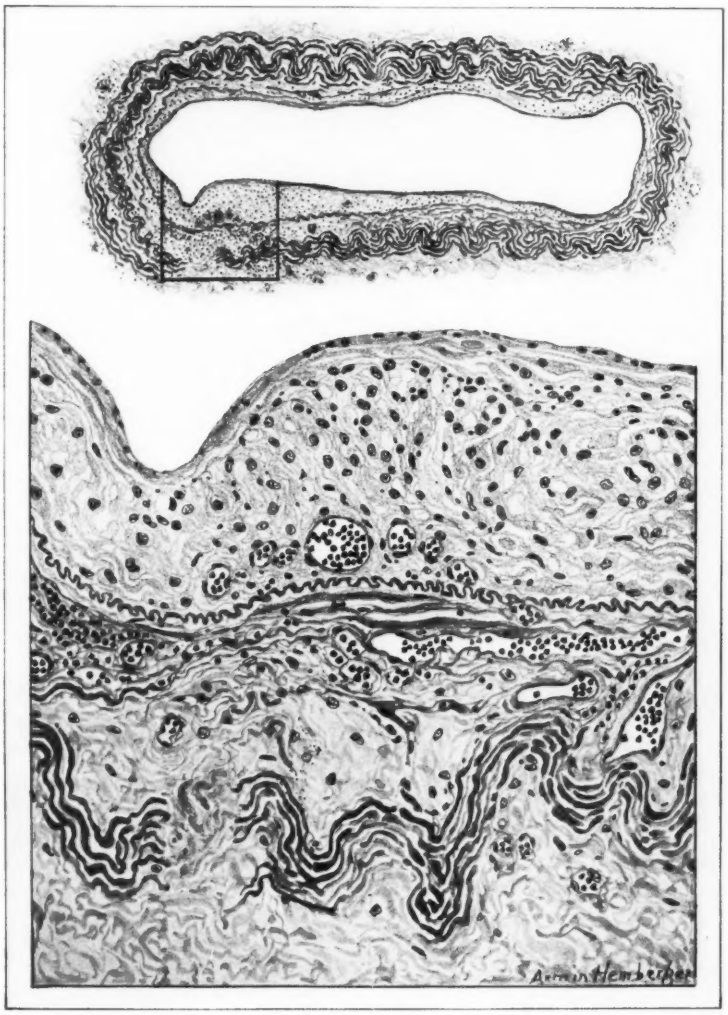

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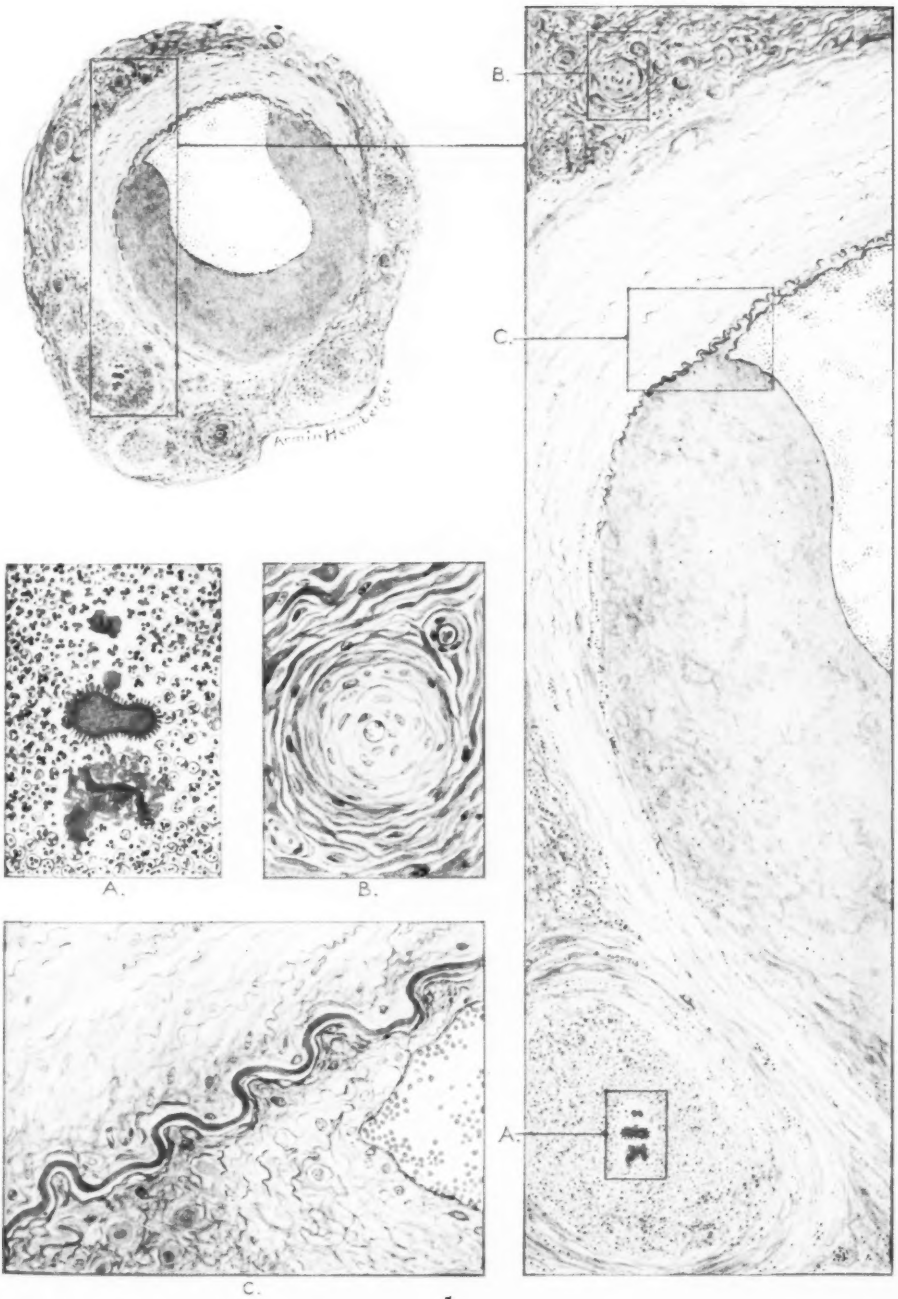
PLATE 4

FIG. 5. Carotid artery of goat No. 13, 6 weeks after injection. Tiny actinomycotic abscesses with dense, fibrous intimal plaque. A = colonies of Actinomyces; B = small adventitial vessels with markedly thickened wall; and C = high power of margin of the intimal plaque. Weigert's elastic tissue and Masson's trichrome stain.

FIG. 6. Carotid artery of a goat 4 days after injection of staphylococci into the wall. Active intimal proliferation (2 mitotic figures in field) is present and a layer of fibrin is seen next to the internal elastic lamella. Masson's trichrome stain.



Winternitz and LeCompte

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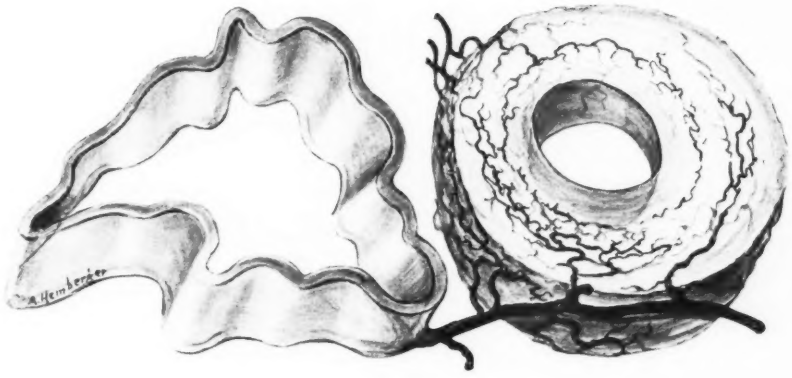
PLATE 5

FIG. 7. Femoral vessels (normal) of a goat injected with India ink by way of a vein. The block was cleared by the Spalteholz method. A retrograde injection of the vasa vasorum of the artery has occurred by way of a small tributary of the vein.

FIG. 8. Block of human femoral vessels injected through the left femoral vein and cleared by the Spalteholz method. There is an extensive injection of the vasa vasorum of both vessels.



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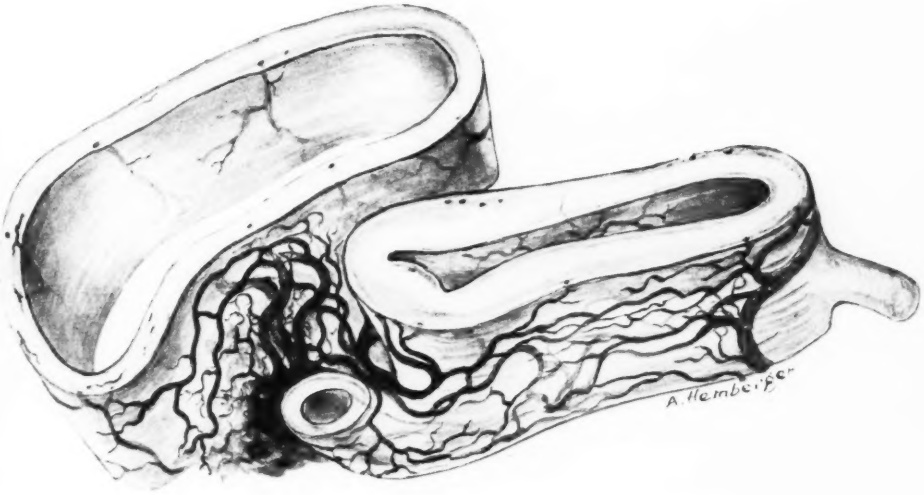

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STUDIES ON INFLAMMATION *

XVIII. On the Mechanism of Leukocytosis with Inflammation

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Recent studies have indicated the presence of a crystalline nitrogenous substance liberated in areas of acute inflammation, capable *per se* of increasing capillary permeability and of inducing prompt leukocytic migration at the site of injury.[1, 2, 3, 4] This substance has been termed leukotaxine. Its liberation and recovery from injured tissue offer thus a reasonable explanation for two of the basic initial sequences in the development of the acute inflammatory reaction. Leukotaxine has been shown to have no manifest physiological property in common with histamine.[1, 3, 4]

Does leukotaxine *per se* induce an increase in the level of leukocytes in the blood stream? Although leukocytosis is well known not to be an invariable accompaniment of an inflammatory reaction, some authors, nevertheless, have expressed the opinion that diapedesis and the level of circulating leukocytes may be referable to one and the same factor.

The observations about to be described indicate that the liberation of leukotaxine evidently bears no relation to the degree of leukocytosis frequently encountered in association with inflammatory processes. Furthermore, the heightened leukocyte level in the blood seems to be referable to the liberation, at the site of injury, of a leukocytosis-promoting factor. The independence of the chemotactic factor from that concerned with leukocytosis is not wholly surprising when it is recalled that certain inflammatory processes characterized by marked leukocytic infiltration can even be accompanied by distinct leukopenia.

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The Effect of Leukotaxine on the Number of Circulating Leukocytes

The initial experiments were performed on rabbits. Blood samples were removed by puncturing the marginal ear vein. White blood counts were performed by the usual technic, utilizing always the same pipette. Repeated counts were made at one-half hour intervals. When, after several preliminary counts, the normal leukocytic rhythm had been established, leukotaxine, extracted as previously described [2] from inflammatory exudates of dogs, was injected into a number of rabbits, either subcutaneously or intravenously. The chemotactic material was always taken up in saline. Its injection was repeated at periodic intervals. A summary of one such experiment is shown in Table I.

Table I

Rabbit Number 18–13, January 25, 1938

Time	Absolute number of leukocytes/mm³	Remarks
10:00 A.M.	9,850	
10:30	9,050	
11:00	8,825	
11:30	7,250	
11:46		50 mg. of leukotaxine (C–D fraction) in 1 cc. of saline injected subcutaneously
12:00 P.M.	9,550	
12:30	9,125	
12:32		Subcutaneous reinjection of 50 mg. of leukotaxine in 1 cc. of saline
1:00	7,100	
1:14		50 mg. of leukotaxine in 1 cc. of saline reinjected subcutaneously; local edema conspicuous
1:30	9,375	
2:00	9,950	
2:30	7,675	
3:00	8,350	
3:30	10,025	
4:00	9,350	
4:30	8,850	
5:00	6,750	Considerable local edema and redness
5:30	9,375	
6:00	7,400	
6:30	7,600	

This experiment indicates that repeated subcutaneous injections of large doses of an active fraction of leukotaxine fail to augment the number of circulating leukocytes. The only manifest effect at the site of injection was considerable local edema, accompanied by redness. The hourly fluctuations in the total white count recall

the type of variations reported previously by Sabin, Cunningham, Doan and Kindwall.[5]

It seemed conceivable, however, that if leukotaxine had any effect on the level of white blood cells this state of affairs might become manifest if the material were introduced in large quantities directly into the circulating blood stream. Consequently, rabbits received repeated intravenous injections of leukotaxine (as a rule fraction C–D)* for 2 to 3 consecutive days. The results of one such experiment are graphically represented in Chart 1, and the data are summarized in Table II.

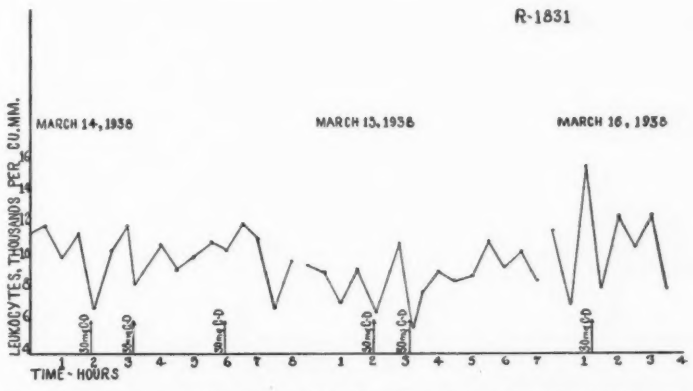

CHART 1. Repeated intravenous injection of 30 mg. of leukotaxine (fraction C–D) into Rabbit 18–31 fails to induce a rise in the leukocyte level above that found as referable to normal rhythmical fluctuations.

The ear at the site of the intravenous injections was fixed in formalin for microscopic study. Examination of the material revealed typical abscess formation consisting of extensive cellular infiltration with numerous degenerated polymorphonuclear leukocytes. At the periphery many uninjured polymorphonuclears were found interspersed among islets of macrophages and fibroblasts.

The results of these experiments, as well as others on dogs (Table VIII), show that intravenous injections of leukotaxine even administered over a prolonged interval of time likewise fail to increase the level of leukocytes in the circulating blood stream.

* Fraction C–D is one of the active purified fractions of leukotaxine, obtained after butyl alcohol treatment of the crude acetone residual fraction.[3]

The conspicuous degree of cellular infiltration and abscess formation at the site of repeated leukotaxine injection bear evidently no direct relation to the number of circulating leukocytes.

TABLE II

Rabbit Number 18–31, March 14–16, 1938

March 14, 1938		March 15, 1938		March 16, 1938	
Time of sampling	Absolute number of leukocytes per mm³	Time of sampling	Absolute number of leukocytes per mm³	Time of sampling	Absolute number of leukocytes per mm³
9:00 A.M.	11,550	9:00 A.M.	10,600	9:30 A.M.	11,700
9:30	11,925	9:30	9,075	10:00	7,150
10:00	10,025	10:00	7,225	10:30	15,725
10:30	11,650	10:30	9,100	10:45	30 mg. of leukotaxine in 1 cc. of saline intravenously injected. There is considerable congestion and dilatation at the point of injection
10:56	30 mg. of leukotaxine in 1 cc. of saline intravenously injected	11:00	Slightly less than 30 mg. of leukotaxine injected intravenously. Right ear shows evidence of dilatation and local congestion in marginal vein		
11:00	6,750			11:00	8,150
11:30	10,350			11:30	12,450
12:00 P.M.	11,925			12:00 P.M.	10,500
12:05	30 mg. of leukotaxine in 1 cc. of saline intravenously injected	11:10	6,500	12:30	12,250
		11:45	10,850	1:00	8,000
12:15	8,300	12:08 P.M.	30 mg. of leukotaxine in 1 cc. of saline intravenously injected	2:57	7,850
12:30	9,175			3:15	Killed by the injection of air into the ear vein
1:00	10,700				
1:30	9,350				
2:00	10,000	12:15	5,425		
2:30	10,800	12:30	7,750		
2:57	30 mg. of leukotaxine in 1 cc. of saline intravenously injected	1:00	9,050		
		1:30	8,525		
		2:00	8,675		
		2:30	10,900		
		3:00	9,450		
3:00	10,450	3:30	10,250		
3:30	12,075	4:00	8,575		
4:00	11,000				
4:30	6,875				
5:00	9,700				

PRESENCE OF A LEUKOCYTOSIS-PROMOTING FACTOR IN INFLAMMATORY EXUDATES

Having apparently eliminated leukotaxine as the factor responsible for the state of leukocytosis frequently associated with inflammation, studies were undertaken in an endeavor to determine

whether the active principle might not be liberated in the exudate as a result of tissue injury.

Danzer [6] showed that the injection in rabbits of various extracts of organs deflects the polymorphonuclear count. The extracts were prepared from normal muscle, liver, brain and testis by shaking the minced tissue in isotonic sodium chloride. Nettleship [7] recently reported experiments on the production of acute inflammation by hemolytic streptococci. The procedure induces leukocytosis in rabbits. This worker correlated the rise in white blood cell counts with the extent of cytoplasmic damage in leukocytes at the site of inflammation. From this type of histological correlation Nettleship concluded that substances released from injured leukocytes are responsible for the developing leukocytosis. Ponder and MacLeod [8] recently expressed the opinion that in the blood stream of rabbits with peritonitis the resultant shift to the left of polymorphonuclear counts is probably referable to the absorption of breakdown products of the cells appearing first in the exudate. These investigators demonstrated that the repeated injection of an irritant into the peritoneal cavity is correlated with a showering into the blood stream of very young polymorphonuclear leukocytes of class I or even of metamyelocytes.

Inflammatory exudates were obtained, as described in earlier studies, from dogs, subsequent to intrapleural injections of 1.5 cc. of turpentine.[9, 10] The duration of the inflammatory reaction prior to thoracentesis varied from 1 to several days. From 15 to 25 cc. of the freshly removed exudate was injected by intracardiac puncture into the circulating blood stream of a normal dog. The sample of exudate was injected, as a rule, immediately into the normal animal. In several instances, however, the exudate was preserved for a few days in the refrigerator without signs of deterioration. To obtain a reasonable basal level prior to the intracardiac inoculation of the exudate, several white blood counts were made on samples of blood obtained by puncturing the cutaneous vessels of the ear. Additional samples were obtained after the administration of exudate, at varying intervals during a period extending from 6 to 8 hours. Throughout the duration of an experiment blood smears were likewise taken and stained by the method of Wright.*

* Repeated injections of exudative material, over a period of several weeks, into

The results of 14 experiments are described in Table III. It is clear from these observations that there is a conspicuous rise in the leukocyte counts several hours after the introduction of an exudate into the blood. The increase in leukocytes averages 70 per cent. It is interesting to note, however, that 1 dog (4–14) showed only an insignificant rise of 25.8 per cent. This dog had been injected with an exudate from an animal that had received 2

TABLE III

Effect of Inflammatory Exudates Injected Into the Circulation on Leukocyte Counts

Number of dog	Absolute number of leukocytes before injection of exudate	Highest level in the absolute number of leukocytes within 6 hours after injection of exudate	Increase in leukocytes
	per cmm.	*per cmm.*	%
4–14	10,525	24,275	130.6
4–27	9,850	20,050	103.5
4–17	7,200	14,000	94.4
4–14	9,975	18,800	88.5
4–17	7,875	14,625	85.7
4–27	14,650	25,550	74.4
4–27	14,950	25,600	71.2
4–14	9,175	14,600	59.1
4–23	8,750	13,725	57.4
4–19	12,100	18,725	54.8
4–14	10,900	16,600	52.3
4–14	8,850	13,100	48.0
4–26	9,050	12,900	42.5
4–14 *	11,425	14,375	25.8
Average	10,377	17,637	70.0

* Exudate obtained from a dog having a profound leukopenia (white blood cells = 3125) resulting from 2 successive injections of turpentine.

successive injections of turpentine. Such drastic treatment with this irritant is often followed by severe leukopenia. At the time of thoracentesis the white count was 3125 leukocytes per cmm. This particular experiment, as well as other but similar observations, suggests that an exudate from an animal with marked leukopenia contains a minimal amount of leukocytosis-promoting factor. The extent of leukocytosis induced by injection of an inflammatory exudate in a normal dog seems, therefore, to be in part at least a

the circulating blood of normal dogs induce degenerative changes in the renal tubules, similar to those seen in nephrosis. These observations will be reported *in extenso* in a separate communication.

function of the degree of leukocytosis present in the animal serving as source of the exudative material.

Several hundred cc. of exudate can often be recovered from the pleural cavity of a dog after a single injection of 1.5 cc. of turpentine. Yet a sample of 15 to 25 cc. of this exudative material introduced into the blood stream of a normal animal induces, as shown above, a definite increase in the number of circulating leukocytes. The bulk of recovered exudate would doubtless considerably dilute the irritant. It seems somewhat doubtful, although not impossible, that the leukocytosis-promoting effect of exudates can be referred to the direct action of turpentine or some of its derivatives.

TABLE IV

Dog 4–27

Time	Number of leukocytes
hrs.: min.	*per cmm.*
8:45	11,200
9:45	12,050
10:42	10 cc. of incubated serum-turpentine mixture injected into circulating blood
10:45	10,850
11:45	12,450
12:45	11,500
1:45	12,850
2:45	12,450
3:45	12,700
5:30	12,300

For this reason an attempt was made to determine the effect of turpentine on the number of circulating leukocytes. Three experiments were performed utilizing the following technic: 10 cc. of blood serum was obtained from a normal dog. 0.5 cc. of turpentine was added to it. The mixture was stirred, incubated at 37° C. for periods varying from 30 minutes to 22 hours, and subsequently injected into the circulating blood of a normal dog. Within the usual interval of study the number of circulating leukocytes failed to rise beyond the level encountered during the normal rhythmical fluctuations. The data of 1 of these experiments are tabulated in Table IV.

The maximum rise in the leukocyte count of a similar experiment, in which turpentine, however, was incubated with serum for

only about a half-hour, was 24.4 per cent. This increment, as indicated in Table V, does not essentially exceed the usual rhythmical fluctuations. A 3rd experiment likewise failed to induce an increase in the number of circulating leukocytes. These results, therefore, render it highly improbable that the effect of exudates

TABLE V

Variation in White Blood Cell Counts in Dogs *

Number of dog	Lowest leukocyte count during a period of 6 hours	Highest leukocyte count during a period of 6 hours	Increase in leukocytes
	per cmm.	*per cmm.*	%
4–19	12,125	17,225	42.1
4–27	12,650	16,500	30.4
4–14	9,050	11,450	26.5
4–23	6,825	8,350	22.3
4–17	9,875	11,525	16.7
4–19	10,775	12,100	12.3
4–27	9,150	10,050	9.8
Average	10,064	12,457	23.8

* Counts were taken every hour.

on the leukocyte level might in any way be referable to a direct action of turpentine *per se* contained in exudates.

As an additional control an attempt was made to obtain exudates with accompanying leukocytosis by irritants other than turpentine. 10 cc. of a saturated solution of magnesium carbonate

TABLE VI

Number of dog	Absolute number of leukocytes before injection of exudate	Highest level in the absolute number of leukocytes within several hours after injection of exudate	Increase in leukocytes
	per cmm.	*per cmm.*	%
4–27	18,550	37,800	103.8
4–27	17,600	30,250	71.9
4–43	19,500	32,850	68.5

containing 1 cc. of staphylococcus toxin was injected intrapleurally. Twenty-four hours later this was followed by a reinjection at the same site of a 1:1 mixture of an aleuronat-starch suspension with coli-bactragen.* The combined irritants induced profuse intra-

* The ingredients of coli-bactragen are gum tragacanth, killed *Escherichia coli*, and aleuronat.

pleural exudation accompanied by marked leukocytosis. The exudative material injected into the blood stream of dogs was followed by a rapid rise in the number of circulating leukocytes. The data of three such experiments are shown in Table VI.

These experiments furnish further support to the view that the leukocytosis-promoting effect induced by exudates is in all probability not due to the presence of turpentine *per se* or of some of its derivatives.*

The changes in the level of leukocytes induced by the injection in a normal dog of about 15 to 25 cc. of exudate are almost of the same order of magnitude as the leukocytosis which develops in dogs with a turpentine-induced intrapleural inflammation. This is illustrated in Table VII. A definite leukocytosis usually develops

TABLE VII

Leukocytosis Associated with Inflammation

Number of dog	Absolute number of leukocytes in the circulation before injection of irritant (turpentine)	Absolute number of leukocytes in the circulation a day after injection of irritant	Increase in leukocytes of circulating blood stream
	per cmm.	*per cmm.*	%
4–29	8,300	21,175	
4–23	7,425	24,975	
4–32	12,300	28,100	
4–21	16,325	25,350	
4–15	9,750	20,100	
Average	10,820	23,940	121.3

about 24 hours subsequent to the introduction of turpentine into the pleural cavity of a dog. A similar effect has been obtained in a few normal dogs by intravascular introduction of about 20 cc. of exudate (Table III, *cf.* Dogs 4–14 and 4–27). In an experiment of this sort, the stimulus on the hematopoietic centers is probably of short duration. On the other hand, with an acute inflammatory reaction in the pleural cavity, there is presumably a continuous

* Further studies with exudates obtained by the intrapleural injection of 10 per cent croton oil in olive oil or by expressing the interstitial exudative material from an extensive burned area of skin on the limb of a dog likewise yielded, when injected in the blood stream, a rise in the number of circulating leukocytes. These observations with different irritating agents serve as additional support to the view that the leukocytosis-promoting effect of exudates is not referable to turpentine or any of its products of degradation (see *Science*, 1939, **90**, 237).

supply of the leukocytosis-promoting factor diffusing into the systemic circulation.

In conclusion, the results summarized in Tables III and VII clearly show that the products of injury liberated in an area of acute inflammation are *per se* capable of inducing in a normal dog a prompt leukocytosis to a degree reasonably comparable with that seen in an animal with an induced pleurisy.

TABLE VIII

Effect of Injections in the Circulation of Leukotaxine, Blood Serum and Broth on Leukocyte Counts

Number of dog	Type of material injected	Absolute number of leukocytes before injection of material	Highest level in the absolute number of leukocytes within several hours after injection of material	Average per cent difference in leukocyte counts
4–27	18 cc. of blood serum	14,150	17,325	
4–14	23 cc. of blood serum	7,175	8,400	
4–14	17 cc. of blood serum	10,800	10,950	
4–17	17 cc. of blood serum	9,950	6,625	
4–19	15 cc. of blood serum	6,175	6,125	
4–29	14 cc. of blood serum	7,100	8,300	
Average		9,225	9,621	+4.3
4–14	75 mg. of leukotaxine (Fraction C–D)	7,700	8,975	
4–17	75 mg. of leukotaxine (Fraction C–D)	7,075	7,025	
4–19	77 mg. of leukotaxine (Fraction C–D)	6,400	6,800	
4–33	100 mg. of leukotaxine (Fraction C–D)	13,800	15,750	
Average		8,744	9,638	+10.2
4–14	23 cc. of broth	9,175	10,550	
4–26	15 cc. of broth culture of an exudate	12,675	9,675	
Average		10,925	10,113	−7.4

The effect on the leukocyte level obtained after the administration of an exudate transcends the maximum rise occurring during the rhythmical leukocytic fluctuations seen in a dog. Such an animal reveals, as a rule, relatively little variation from hour to hour. During the period of experimentation, *i.e.* 6 to 8 hours, the maximum increase in leukocytes of several normal dogs averaged 23.8 per cent (Table V). The counts were made on dogs which,

on separate days, had been injected with exudates (Table III). A comparison of Tables III and V indicates that there is therefore, roughly, as a result of a single intravascular injection of exudate, a threefold increase in the leukocyte level of the circulation.

To extend the observations, dog serum, sterile broth, large doses of leukotaxine in saline and a broth culture of an exudate were injected directly into the circulating blood of normal animals and of dogs previously receiving exudates. The results of these ex-

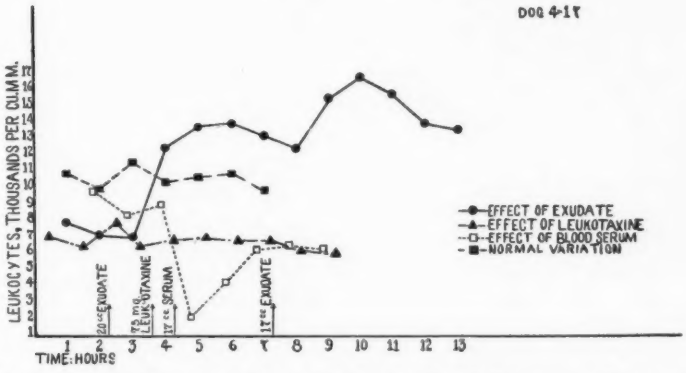

CHART 2. The rise in the blood leukocyte level of Dog 4–17 after intravascular injection of an inflammatory exudate. Note that subsequent reinjection of the exudate induced a further rise in the leukocytic level. Compare this effect with the ineffectiveness of leukotaxine or of blood serum. The normal variations in the white count over a period of 7 hours are likewise indicated.

periments are summarized in Table VIII. A leukocytosis invariably failed to develop in these experiments. The intravenous administration of dead bacteria (broth culture of killed *Staphylococcus aureus*) likewise proved incapable of altering significantly the total number of leukocytes.

Type experiments are graphically represented in Charts 2 and 3. When the exudate is reinjected in a dog several hours following a 1st injection, the primary effect can be reinforced and a secondary rise in the level of leukocytes may become manifest (Chart 2). The transitory leukopenia immediately following the intravascular injection of serum (Chart 2) or even of exudate (Chart 3) recalls the leukopenia described by Ewing [11] after intravenous injection

of bacteria, or by Webb [12] during anaphylactic shock. Doan and his collaborators [13] likewise described an initial leukopenia following the injection of sodium nucleinate in rabbits. This phenomenon has been explained as a trapping of leukocytes in the lungs, liver or spleen. The administration of a cell-free exudate in an otherwise normal dog is at least as effective in inducing a leuko-

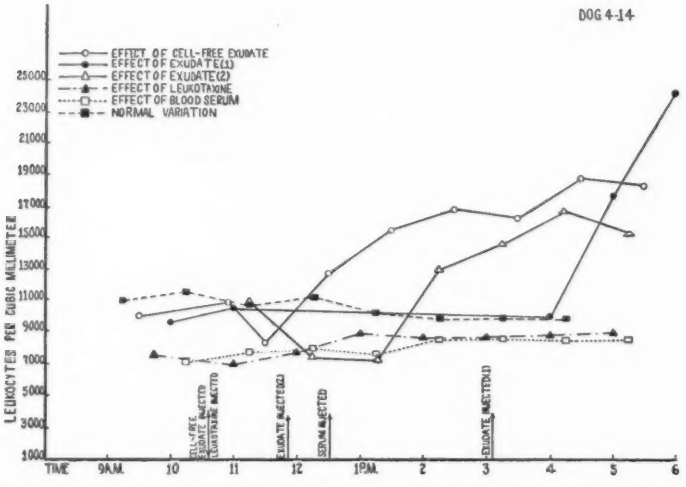

CHART 3. The rise in the blood leukocyte level of Dog 4–14 after intravascular injection of an inflammatory exudate. The sample of exudate (1) was injected in the middle of the afternoon. The rise in the leukocyte level was noted about an hour later. Sample (2) was administered in the forenoon. A definite rise in the leukocyte level was apparent about 2 hours later. The cell-free exudate was found to be at least as effective in inducing leukocytosis as the whole exudate. Blood serum or 75 mg. of leukotaxine (shown by the dotted or by the dash and dotted lines) was apparently ineffective in inducing a rise in the number of white blood cells.

cytosis as the whole exudate (Chart 3). This fact, in addition to the occasional transitory leukopenia following the injection of an exudate, would rule out the rise in the leukocyte level as due to a mechanical transfer of cells contained in the injected exudates.

The leukocytosis-promoting factor of exudates is thermolabile. Boiling the exudate, or merely heating it for several hours at 60° C., inactivates it. This is illustrated in the experiments listed in Table IX. The factor is essentially non-dialyzable. When dialyzed against running water (tap) through a cellophane mem-

brane for about 24 hours the indiffusible residual material retains a considerable amount of its potency (Table IX). Heating the residual fraction of the dialyzed exudate to 60° C. completely inactivates its potency. The results of these observations are illustrated in Chart 4.

The leukocytosis-promoting factor seems to exert its effect primarily on the hematopoietic tissue in the bone marrow. Follow-

TABLE IX

Effect of Heat and of Dialysis on the Leukocytosis-Promoting Factor

Number of dog and material injected into the blood stream	Absolute number of leukocytes before injection of exudate	Highest level in the absolute number of leukocytes within 6 hours after injection of exudate	Increase in leukocytes
	per cmm.	*per cmm.*	%
4–14. Exudate heated to boiling	8,750	9,050	3.4
4–27. Exudate heated to 60° C. Maintained for several hours at that temperature	9,800	10,350	5.6
4–27. Exudative material retained in cellophane bag after dialysis	14,850	22,200	49.5
4–27. Retained material after dialysis and heated to 60° C. for several hours	12,400	12,700	2.4
4–23. Retained exudate after dialysis	9,525	26,375	56.5
4–14. Retained exudate after dialysis	10,775	15,925	47.8

ing the injection of an exudate there is a definite rise in the number of immature polymorphonuclear leukocytes. The data from a type experiment (Table X) support this view.

A survey of the figures in Table X clearly indicates that the absolute number of mature polymorphonuclear leukocytes barely increases after the administration of the exudate. On the other hand, the absolute number of immature polymorphonuclear leukocytes has augmented over sevenfold. Although classical Arneth counts were not consistently performed on blood smears, careful examination for the presence of filaments and the type of lobulation

readily differentiated mature from immature forms of polymorphonuclear leukocytes. This is exemplified in Figures 1 and 2. The results indicate therefore that the leukocytosis-promoting factor evidently exerts its stimulating action on the bone marrow, favoring

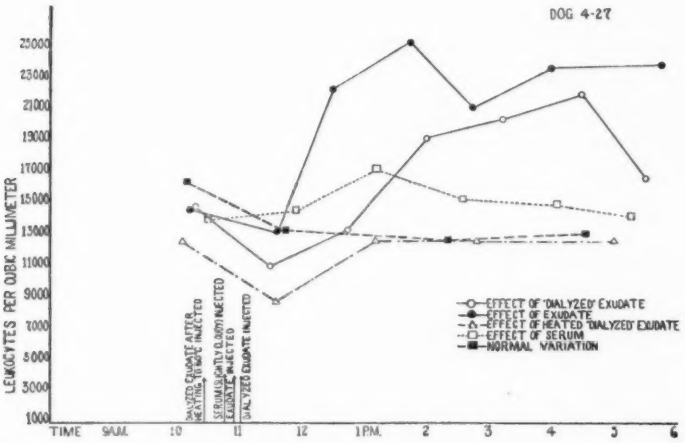

CHART 4. A comparison of the effect of a sample of exudate on the blood leukocyte level of Dog 4–27 with that obtained with the residual dialyzed fraction of a sample taken from the same exudate. Note that the material retained in the cellophane bag after prolonged dialysis has, to a considerable degree, retained the leukocytosis-promoting factor. Heating this residual dialyzed fraction to 60° C. completely inactivates the contained factor (dash and dotted line). The effect of blood serum and the normal variations in the counts of this animal are likewise indicated.

thus the so-called "shift to the left" in the differential leukocytic picture of the circulation. This effect on the granulocytes is precisely similar to the well known type of response elicited by the presence of a focus of inflammation accompanied by leukocytosis.[8]

The nature of the leukocytosis-promoting factor is as yet unknown. Studies are now in progress in an endeavor to identify it. Some of its properties (*e.g.* thermolability, indiffusibility) are not inconsistent with the belief that it may prove to be a protein or perhaps a degradation product of protein catabolism.*

* Studies in progress since the completion of this article indicate either that the leukocytosis-promoting factor is a globulin or that at least it seems associated with the globulin fraction of exudate. These observations will be reported *in extenso* in a forthcoming article.

In 1897 Ames and Huntley [14] reported observations on the development of leukocytosis by nucleinic acid. Ewald [15] showed that yeast and thymus nucleinic acid, guanine and xanthine induce leukocytosis in dogs. Fox and Lynch [16] found that nucleinic acid causes at first a fall in the number of polymorphonuclear leukocytes, followed later by a rise beginning after the 7th hour. Ponder and his collaborators have studied for a number of years the effect of various substances on the Arneth count. This investigator showed that thyroid extract, injected into rabbits, causes an outpouring of relatively immature granulocytes (*i.e.* classes I and

TABLE X

Dog Number 4-27

1. Absolute number of leukocytes prior to injection of exudate	14,650/mm³
(*a*) Percentage of polymorphonuclear leukocytes	86
(*b*) Absolute number of polymorphonuclear leukocytes	12,599/mm³
(*c*) Percentage of immature polymorphonuclear leukocytes	8
(*d*) Percentage of mature polymorphonuclear leukocytes	92
(*e*) Absolute number of immature polymorphonuclear leukocytes	1,008/mm³
(*f*) Absolute number of mature polymorphonuclear leukocytes	11,591/mm³
2. About 20 cc. of exudate injected into blood stream	
3. 5 hours later: absolute number of leukocytes	23,825/mm³
(*a*) Percentage of polymorphonuclear leukocytes	88
(*b*) Absolute number of polymorphonuclear leukocytes	20,960/mm³
(*c*) Percentage of immature polymorphonuclear leukocytes	38
(*d*) Percentage of mature polymorphonuclear leukocytes	62
(*e*) Absolute number of immature polymorphonuclear leukocytes	7,968/mm³
(*f*) Absolute number of mature polymorphonuclear leukocytes	12,998/mm³

II).[17] Climenko [18] demonstrated that parenteral administration of irradiated ergosterol deflects the Arneth count. This effect is similar to that reported previously by Kennedy and Thompson [19] on the influence of ultraviolet irradiation on the Arneth count. Ponder and Flint [20] showed that in the rabbit gelatin, trypsin, colchicine, nucleic acid and thyroxin, all deflect the Arneth count. Bennet and Drury [21] found that adenosine induces in the rabbit a leukopenia followed by a leukocytosis. The increase in cells was shown to be primarily due to an outpouring in the circulation of polymorphonuclear leukocytes. Sabin [22] expressed the opinion that nucleic acid is most likely involved in the normal mechanism, calling forth leukocytes from the bone marrow. She based her view on a number of studies, particularly those of Löwit [23] who found that a leukopenia is followed by a leukocytosis after the injection of

numerous substances including bacterial proteins and nucleic acid; and also on the experiments of Doan, Zerfas, Warren and Ames,[13] who showed that following large doses of nucleic acid in the rabbit there develops an initial leukopenia followed ultimately by a leukocytosis. The spleen is, according to these investigators, solely responsible for the temporary depression of white cells in the circulation. Splenectomy favors the production of a leukocytosis without the preliminary leukopenic phase. Furthermore, the injection into normal rabbits of adenine and guanine nucleotides, which are split products of nucleic acid, induces immediate leukocytosis. Finally Hoffmann [24] found in rabbits that adenylic acid,

TABLE XI

Number of dog	Approximate amount of yeast nucleic acid injected	Absolute number of leukocytes per cmm. at varying intervals before and after injection of nucleic acid				
		Before injection	1–2 hrs. after injection	3–5 hrs. after injection	5–7 hrs. after injection	20–26 hrs. after injection
4–33	17 mg.*	15,700	6,150	11,900	10,750	14,500
4–14	25 mg.	11,700	9,850	10,975	11,125	8,450
4–14	200 mg.	8,825	2,275	3,975	8,550	15,525
4–33	1000 mg.**	17,500	4,750	14,500	18,900	30,400

* This concentration of nucleic acid represents 1 mg. per kilo of body weight.
** This figure is an approximation, inasmuch as the solution was supersaturated.

in contrast to guanylic acid, causes a marked leukocytosis. The pyrimidine nucleotides were found to be ineffective.

The foregoing summary of earlier work indicates that nucleic acid or some of its derivatives is evidently capable of stimulating the bone marrow to discharge immature polymorphonuclear leukocytes into the circulation. It is reasonable to suppose that with tissue injury, nucleic acid derivatives might be liberated in abundance in an inflamed area. For this reason experiments were undertaken with varying concentrations of nucleic acid to determine whether or not results on the leukocytic level similar to those obtained with exudates could be duplicated with this acid.

Dogs were accordingly injected into the heart with various concentrations of yeast nucleic acid in solutions ranging from 1 mg. per kilo to 20 cc. of a 5 per cent suspension. The results of these experiments are summarized in Table XI.

The results shown in Table XI indicate that with low doses of nucleic acid there is no significant effect on the absolute number of circulating leukocytes. With high concentrations of the acid,

however, in agreement with the earlier studies of Fox and Lynch,[16] there is a preliminary leukopenia accompanied by symptoms of toxicity. This is followed by a beginning rise in the cell count from about the 6th or 7th hour. The cellular increase progresses, producing about 24 hours later a state of frank leukocytosis. This retarded development of leukocytosis is unlike the relatively prompt response induced with exudates (*cf.* Table III). It is, however, conceivable that derivatives of nucleic acid, such as adenine or guanine nucleotides, might produce an accelerated leukocytosis. This, as mentioned above, has been shown by Doan and his collaborators to occur when normal rabbits are injected with these compounds.[13] The injection of 6 mg. of adenosine (which is essentially a nucleoside of adenine) in saline into the circulating blood of a dog fails to induce prompt rise in the level of leukocytes. The possibility is not precluded, however, that inflammatory exudates may contain considerable quantities of nucleotides which *per se* can account for the induced leukocytic response.* Studies are now in progress to test this possibility.

Moon [25] is of the opinion that histamine or the so-called hypothetical H-substance is of definite significance in the production of leukocytosis associated with inflammatory conditions. This worker finds an increased leukocyte count in cats following the intravenous injection of 1 to 2 mg. of histamine. The injection of similar or slightly higher amounts (*e.g.* 5 mg.) in dogs is, in my experience, wholly ineffective in altering appreciably the total leukocyte count. When 8 to 12 mg. of histamine dihydrochloride are injected into the blood stream of a dog a very slight rise in the level of leukocytes may become manifest. Such concentration of histamine is, however, much higher than the amount recovered from exudate containing the active leukocytosis-promoting factor. To verify this further, histamine was extracted by the method of Barsoum and Gaddum [26] from two samples of exudate, of 23 cc. and 40 cc. respectively. The end-product in either case failed to induce any rise in the leukocyte level of the circulation. Yet the exudates, *per se*, contained the leukocytosis-promoting factor.

* A solution of pentnucleotides was obtained through the courtesy of the Smith, Kline and French Laboratories, Philadelphia, Pa. This product is a mixture of sodium salts of pentose nucleotides. Fifteen or 20 cc. of this material injected in dogs failed to induce, in the usual interval, a rise in the number of circulating leukocytes.

These observations would tend to rule out histamine as an important element in explaining the leukocytosis-promoting effect of exudates.

Conclusions

Leukotaxine, a crystalline nitrogenous substance recovered from inflammatory exudates, and known to increase capillary permeability and cause diapedesis of polymorphonuclear leukocytes, fails to induce an increase in the leukocytic level of the circulation in either the dog or the rabbit. Hence it can scarcely be responsible for the leukocytosis of inflammation.

There is present in exudates a leukocytosis-promoting factor capable of inducing a rise in the leukocyte level of dogs. This is demonstrable when either the whole or the cell-free exudate is introduced into the circulating blood.

An inflammatory exudate removed from an animal with leukopenia and injected into a normal dog fails, as a rule, to induce a marked rise in the leukocyte count.

Blood serum, sterile broth, bacterial cultures of exudates and cultures of killed bacteria (*Staphylococcus aureus*) are all ineffective in causing an increase in the leukocyte level of the circulation.

Histamine and adenosine are likewise essentially inactive. Extraction of exudates containing the active leukocytosis-promoting factor for histamine, yields an end-product incapable of inducing leukocytosis. Nucleic acid injected into the blood stream in large concentration favors, after a considerable latent period, a rise in the number of leukocytes. This delayed effect differs from the prompt response induced by exudates.

The action of the leukocytosis-promoting factor seems to be primarily on the bone marrow, producing an outpouring of immature granulocytes into the circulation. The factor is thermolabile. Heating the exudate to 60° C. inactivates the material. The factor, after prolonged dialysis of the exudate through a cellophane membrane, is found to be, in large part, indiffusible. Further studies are now in progress in an endeavor to identify the nature of the leukocytosis-promoting factor present in inflammatory exudates.

Note: I am greatly indebted to Mr. M. A. Kadish for drawing the charts.

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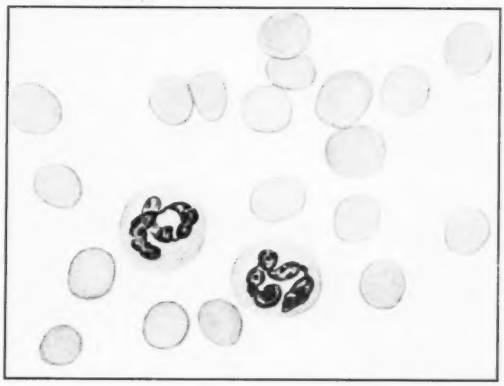
DESCRIPTION OF PLATE

PLATE 6

FIG. 1. Drawing of blood smear taken from Dog 4–27 before the intravascular administration of an exudate. Compare the mature forms of polymorphonuclear leukocytes as indicated by the type of multilobulations and the presence of filaments with the immature non-filamentous cells shown in Fig. 2.

FIG. 2. Drawing of blood smear taken from Dog 4–27 after the injection of an exudate. The immature non-filamentous cells are conspicuous. The absolute leukocyte count before administration of the exudate was 14,950 per cmm., whereas 4½ hours after injection of the exudate the number of leukocytes attained a level of 25,600 per cmm. The outpouring of immature granulocytes in the circulation supports the view that the leukocytosis-promoting factor probably acts primarily on the hematopoietic centers in the bone marrow. × 1300.



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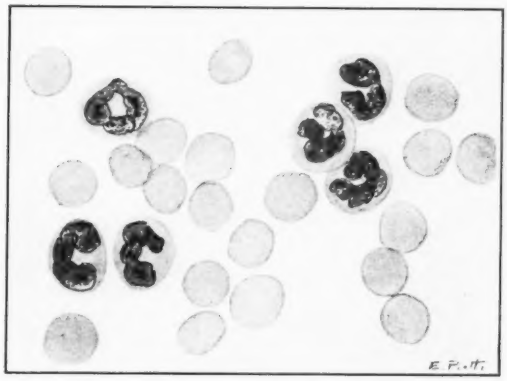

2

ORIGIN OF PERINEURAL FIBROBLASTOMA *

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The crux of the discussion concerning the common encapsulated tumor of peripheral nerves centers about the nature of the type cell — whether it is a fibroblast of the connective tissue sheath (Mallory,[1] Penfield [2]), or whether it is of Schwann cell origin (Verocay,[3] Masson [4]). The former view rests on the morphological resemblance of the tumor cells to fibroblasts and the occurrence of a reticulum stroma similar to that of the connective tissue sheath of the nerve fiber. Mallory called these tumors perineural fibroblastoma, a term which was accepted by Penfield who recognized, however, that they might be derived from endoneurium as well as perineurium. The schwannian hypothesis is based largely on Masson's belief in the essential similarity of these tumors to experimental schwannomas produced in rabbits. Evidence for this view is entirely indirect since specific methods for the staining of Schwann cells were not employed. It was because of the uncertainty as to which of two components of peripheral nerves (fibroblasts or Schwann cells) gave rise to these tumors that Stout [5] introduced the term neurilemoma, by which he would indicate a derivation from either the Schwann cell or endoneurium. The objection to this designation becomes apparent when we consider the established usage of the term neurilemma, which refers to the membrane of Schwann although some authors have used it to indicate the endoneurium. Stout would broaden the term to include both the Schwann membrane and the endoneural sheath. Such liberty in the use of the term neurilemma seems unjustified in view of its established usage, and the designation neurilemoma, therefore, would appear to be unwarranted and confusing. These tumors have been called neurinomas (Verocay [3]) which means tumors of nerve fibers. Since it is agreed that these are not tumors of nerve fibers, the term neurinoma should be discarded.

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As far as we can ascertain, this is the first demonstration by direct staining methods that the type cell of these tumors is not the Schwann cell. Two perineural fibroblastomas of the vagus nerve roots will be discussed, the first, to our knowledge, that have been recorded. Study of the two tumors in serial section has presented the opportunity of making observations pertinent to the question in regard to the particular connective tissue sheath from which these tumors arise.

The method employed for the demonstration of Schwann cells was Dockrill's modification [6] of Hortega's silver impregnation technic. This method requires fixation in formalin-urea-potassium iodide and staining with undiluted silver carbonate. This technic, similar to other silver methods, is not entirely reliable, but it gives fair results in a high percentage of trials if the material is fresh and if the staining is done within 6 to 24 hours after fixation. It is imperative that the material be perfectly fresh and it must be placed in the fixative promptly after removal from the body. Schwann cells may be distinguished clearly from fibroblasts in preparations impregnated according to this method (Fig. 1).

By this technic Schwann cells have been impregnated along nerve fibers in a majority of trials (Fig. 2), but it has never been possible to reveal Schwann cells within any of the twelve perineural fibroblastomas examined. The cells impregnated by this technic in the perineural fibroblastomas presented clear-cut features of fibroblasts (Fig. 3). In a case of neurofibromatosis of the von Recklinghausen type, Schwann cells were clearly impregnated along nerve fibers which passed through the tumor, whereas the tumor cells themselves proved to be of a totally different type (Figs. 4 and 5). The Schwann cells were rectangular, with large, clear oval nuclei containing scant chromatin particles and with considerable spongy cytoplasm which was often vacuolated; the tumor cells, on the other hand, similar to those in the solitary perineural fibroblastomas, were narrow and elongated with deeply staining nuclei and cytoplasm which ended in tapering processes, one at each pole of the cell, the entire cell presenting the appearance of the fibroblast. In some areas where degenerative changes had occurred the cells were round or irregular in shape with several straight or wavy processes radiating from the cell body. Similar cells were disclosed in the loose reticular areas of the soli-

tary perineural fibroblastoma. In no respect, however, did any of the tumor cells present the morphological appearance suggestive of the clearly impregnated Schwann cells, which occurred along normal nerve fibers or in traumatic neuromas (Figs. 7, 8 and 9). Around the nuclei of the Schwann cells which occurred along nerve fibers in the neurofibroma of the von Recklinghausen type, the toluidine blue technic of Reich, which requires fixation in Müller's fluid, revealed numerous purple granules (protagon-like or π granules of Reich), so characteristic of Schwann cells (Fig. 6). Such granules did not occur within the tumor cells.

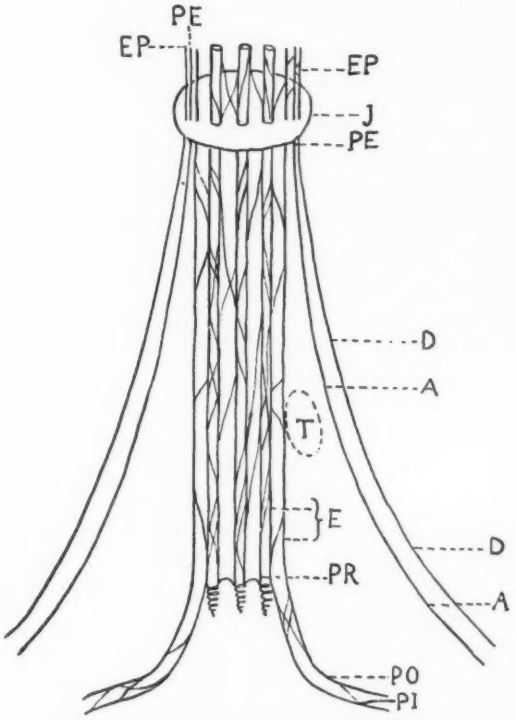
Both tumors of the vagus nerve roots were found during the course of routine autopsies on adults. One was from a male aged 60 years, who died from carcinoma of the prostate gland with extensive metastases. The other tumor was from a patient who died from a non-neoplastic disease, the exact nature of which is not known. No symptoms which might be attributed to the presence of the tumor occurred in either case.

The tumor in each case occurred on one of the cephalic, smaller ventral roots of the vagus nerve. The larger tumor (Figs. 10, 11 and 12) measured 2 mm. in diameter, the other tumor being about one-half this size. Both tumors were well encapsulated and arose from the peripheral non-glial segment of the nerve root about 5 mm. from the site of origin of the nerve from the brain stem. It will be recalled that at this point the arachnoid has not yet become applied to the nerve root as the perineurium; this occurs more peripherally along the course of the nerve root in the vicinity of the jugular foramen (Text-Fig. 1).

Microscopically in each case the tissue presented the appearance of interlacing bundles of cells (Figs. 13 and 14) and reticulum fibers which were cut across at various angles so that certain groups of nuclei appeared round and others oval or elongated. The nuclei contained moderate amounts of evenly dispersed chromatin granules without nucleoli. They were surrounded by a vaguely fibrillar cytoplasm which usually ended in two long processes, one at either pole of the cell. There was some tendency toward palisading of nuclei. An occasional nucleus was large and bizarre in shape, containing a nuclear inclusion body. Mitotic figures did not occur. Preparations stained by Laidlaw's method for connective tissue revealed an abundant connective tissue stroma which consisted of

delicate reticulum fibers (Fig. 15). Moderate numbers of thin walled blood vessels occurred.

The tumor in each case arose from the peripheral border of the



TEXT-FIG. 1. Diagram showing the relation of the tumor (T) in each case to the connective tissue sheaths of the nerve. J = the jugular foramen; PR = the pial ring formed by the inner layer of pia mater. PI, together with the outer layer of the pia mater, (PO) forms the endoneurium (E); PE = the perineurium which is the peripheral extension of the arachnoid (A). EP, the epineurium, is the peripheral extension of the dura mater (D).

nerve root. Nerve fibers were chiefly embedded in the capsule of the tumor but study of serial sections revealed occasional myelinated nerve fibers well within one of the tumors (Fig. 16).

Discussion

There is considerable evidence that Schwann cells are homologous with oligodendroglia, and the fact that the Dockrill technic brings out both types of cells quite clearly is a point rather in favor of this. Of more importance in favoring this homology is the fact that the cells possess a morphological resemblance to each other, and along the course of the nerve fibers from the central to the peripheral nervous system cells are observed which appear to be transitional between oligodendroglia and Schwann cells. Oligodendrogliomas are not associated with reticulum of the type noted in the tumors under consideration and, on the basis of this homology, one would not expect real schwannomas to be associated with such reticulum. This is a bit of indirect evidence against the schwannian origin of perineural fibroblastoma. In this study, however, it is the direct evidence against the schwannian hypothesis that is stressed.

The occurrence of π or protagon granules within Schwann cells, and their absence within the type cells of the tumor, do not exclude a Schwann cell origin for these tumors. The granules have been found to be related to the presence of myelin along nerve fibers, and hence their absence might merely indicate the absence of myelination. However, the inability to impregnate Schwann cells in solitary perineural fibroblastomas, while under the same conditions Schwann cells have been impregnated along peripheral nerve fibers, constitutes evidence against the schwannian origin of these tumors. Further convincing evidence is to be found in the clear impregnation of Schwann cells along the course of a nerve fiber within an encapsulated tumor in a case of von Recklinghausen's neurofibromatosis in which the tumor cells proved to be of a totally different type, presenting the characteristics of fibroblasts. Since the type cell of the solitary perineural fibroblastoma and the neurofibroma of the von Recklinghausen type are doubtless the same, the totally different character of the tumor cells and the Schwann cell, as seen in a single microscopic field in a case of neurofibromatosis, seems to be valid evidence against a schwannian origin for the tumors in each instance.

One might argue that the tumor cells might still represent proliferating Schwann cells and yet not be impregnated by the Dock-

rill technic. It has been possible to answer this objection effectively by impregnating proliferating Schwann cells in a traumatic neuroma of the ulnar nerve of 7½ months duration by this technic. The Schwann cells thus stained occurred both along nerve fibers and independent of nerve fibers, at times in groups of two or three nuclei within the same cytoplasm (Fig. 8). They possess large, round or oval nuclei with perinuclear spongy cytoplasm which usually becomes attenuated a relatively short distance from the nuclei. The Schwann cells even in proliferation possess distinctive characteristics and stained by the Dockrill technic they may be quite clearly differentiated from the elongated fibroblasts (Fig. 9). The type cell of the perineural fibroblastoma, or of the neurofibroma of the von Recklinghausen type, possesses no features even remotely suggestive of normal or of proliferating Schwann cells.

The occurrence of cells resembling neuroglial cells within the loose reticular areas of perineural fibroblastoma (particularly noticeable in preparations stained by Dockrill's technic) makes it understandable that these tumors were formerly thought to contain neuroglial elements. However, these cells possess no gliosomes and present no vascular or connective tissue attachments. Moreover, they occur within an abundant reticulum stroma which is not associated with neuroglia. Their appearance is only superficially similar to neuroglial cells. They occur only in areas where the tissue structure is lax. Transitions may frequently be traced from the elongated fibroblasts to these multipolar cells, some of which are bizarre in shape. These cells arise as a result of degenerative changes affecting the tumor cells (fibroblasts).

The fibroblastic nature of perineural fibroblastoma seems evident on the basis of the cell type and the stroma of the tumor. The rich reticulum stroma recalls that of endoneurium. Attempts have been made to demonstrate that reticulum is not necessarily related to fibroblasts but may be formed by other types of cells, for example Schwann cells, but these attempts have not been convincing. Masson, as evidence to support the view that Schwann cells may provoke the formation of reticulum without the intervention of fibroblasts, cites Nageotte's demonstration that reticulum occurs along the central prolongations of nerve fibers through the pial ring in the same distribution as the mem-

brane of Schwann. Nageotte [7] was of the opinion that fibroblasts did not occur in this region and hence were not essential for the formation of reticulum. However, this argument is not valid (Tarlov [8]) since I was able to demonstrate fibroblasts central to the pial ring, along the same distribution as reticulum (Figs. 17 and 18). The fibroblast is constantly found in association with reticulum of the endoneurium.

The structure of the tumors of the vagus nerve roots was characteristically that of perineural fibroblastoma. The occurrence of the two tumors on portions of nerve roots which were unaccompanied by perineurium indicates that the endoneurium must have given rise to the tumors. Therefore, the term endoneural fibroblastoma would appear to be a more accurate designation for these tumors. If such tumors arose from perineurium, one might expect to find them on the optic nerve which contains perineurium but not endoneurium. To my knowledge, however, no such tumor, arising unmistakably from the optic nerve, has ever been reported. It has been stated, however, that such tumors may arise from the perineurium (Bailey and Herrmann [9]). If one accepts this view and is inclined to use a term that is exact in its connotation, then endoperineural fibroblastoma would appear to be the best designation for these tumors. The term perineural fibroblastoma should be retained for these tumors although it must be understood that they commonly arise from the endoneurium.

It has been stated [2] that the solitary perineural fibroblastoma may be differentiated from the neurofibroma of the von Recklinghausen type by the fact that the former tumor has nerve fibers about its capsule but not within the tumor itself, the occurrence of nerve fibers within the tumor being considered characteristic of the multiple neurofibromas. While this view would seem to be true in general, the observation of an occasional nerve fiber within one of the solitary tumors, which is reported, would indicate that exceptions to the rule may occur.

Summary and Conclusions

1. Direct staining evidence by a technic that brings out normal and proliferating Schwann cells excludes the Schwann cell as

the type cell of the common encapsulated tumor of peripheral nerves.

2. The type cell of these tumors presents the morphological characteristics of the fibroblast.

3. The term perineural fibroblastoma should be retained for these tumors although it must be realized that they may arise from endoneurium, as in the case of the tumors of the vagus nerve roots which are herein reported.

4. Although the occurrence of nerve fibers within an encapsulated tumor of the peripheral nerves is usually characteristic of multiple neurofibromatosis, an occasional nerve fiber may occur within the solitary perineural fibroblastoma.

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DESCRIPTION OF PLATES

PLATE 7

FIG. 1. Schwann cell (S) along the oculomotor nerve with fibroblast (F) just out of focus. Note the clear contrast between these cells. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 1400.

FIG. 2. Schwann cell along the oculomotor nerve showing less vacuolization than in Figure 1. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 1200.

FIG. 3. Type cell (fibroblasts) of perineural fibroblastoma. Note the clear contrast between these cells and the Schwann cells in Figures 1 and 2. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 1200.

FIG. 4. Schwann cell (S) along a nerve fiber within a tumor of the von Recklinghausen type. Note the contrast between the Schwann cell and the elongated cells (fibroblasts, F) which form the type cell of this tumor as well as of the perineural fibroblastoma. Silver carbonate impregnation (Dockrill's modification of the Hortega's technic). × 1470.

FIG. 5. Same as in Figure 4. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 2000.

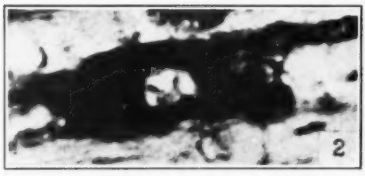
FIG. 6. Protagon (π) granules along a nerve fiber within a neurofibroma of the von Recklinghausen type. Reich's toluidine blue stain. × 1850.

FIG. 7. Schwann cell (S) occurring along a nerve fiber in a traumatic neuroma of the ulnar nerve. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 1600.

FIG. 8. A group of proliferating Schwann cells occurring in a traumatic neuroma of the ulnar nerve. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 1600.

FIG. 9. Schwann cells (S) and fibroblast (F) in a traumatic neuroma of the ulnar nerve. Note the clear contrast in cell types. Silver carbonate impregnation (Dockrill's modification of Hortega's technic). × 1600.

S
F
1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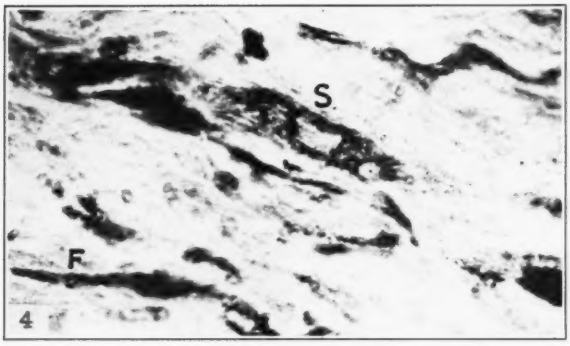
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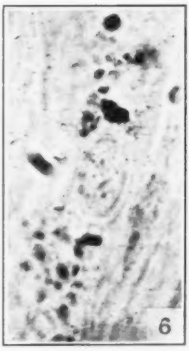
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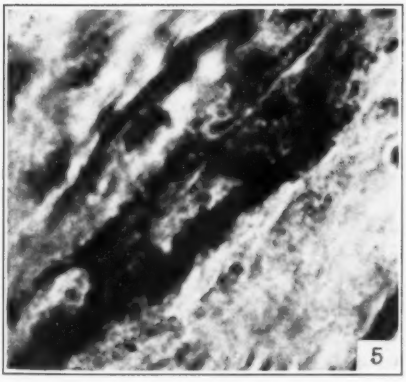
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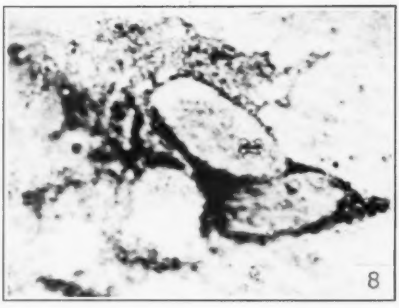
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PLATE 8

FIG. 10. Photograph showing a perineural fibroblastoma along one of the roots of the vagus nerve in a 60 year old male.

FIGS. 11 and 12. Drawings of the tumor shown in Figure 10.

FIG. 13. Microphotograph of the same tumor shown in Figure 10. The appearance is characteristic of perineural fibroblastoma. Hematoxylin-eosin stain. × 100.

FIG. 14. Higher power of tumor shown in Figure 13. Hematoxylin-eosin stain. × 925.

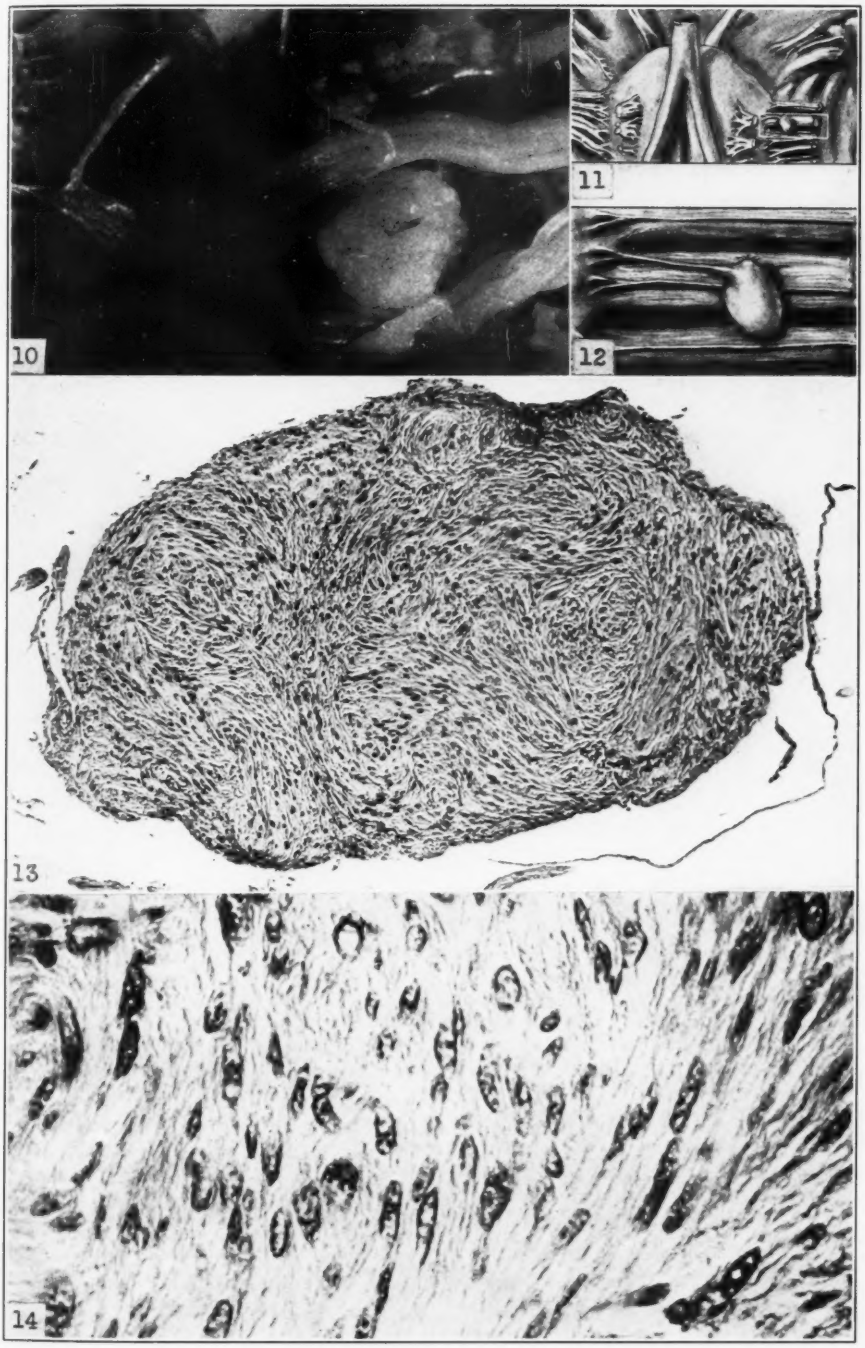
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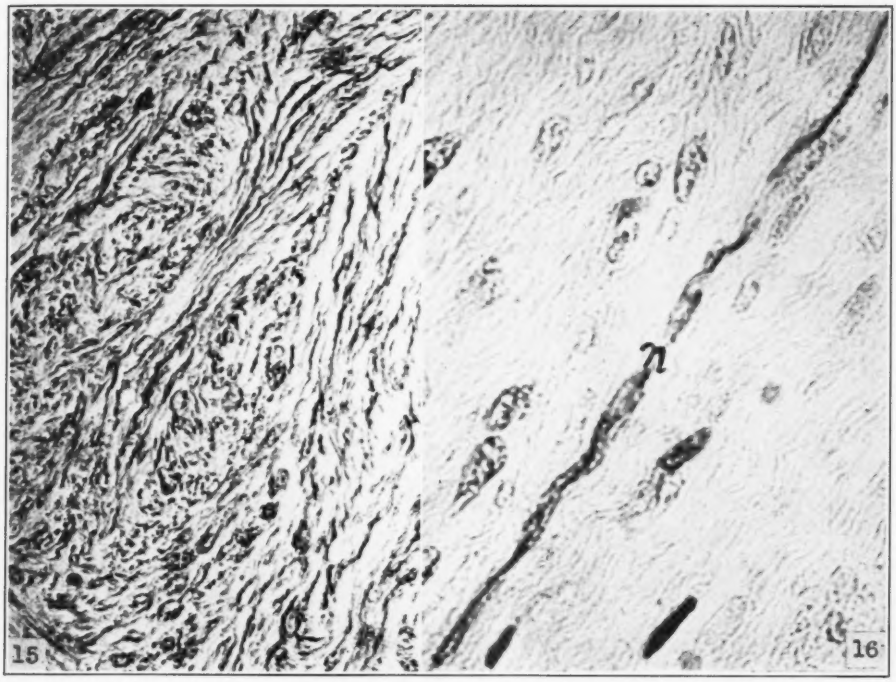
PLATE 9

FIG. 15. Reticulum stroma of the tumor shown in Figure 13. Laidlaw's modification of Hortega's technic. × 800.

FIG. 16. Nerve fiber (n) coursing through the tumor shown in Figure 15. Loyez' myelin sheath stain. × 1000.

FIG. 17. Reticulum occurring along nerve fibers central to the pial ring. Laidlaw's modification of Hortega's technic. × 750.

FIG. 18. Fibroblast (F) occurring just central to the pial ring (indicated by dotted line) in the same region of distribution as the reticulum tubules shown in Figure 17. Hematoxylin-eosin stain. × 1000.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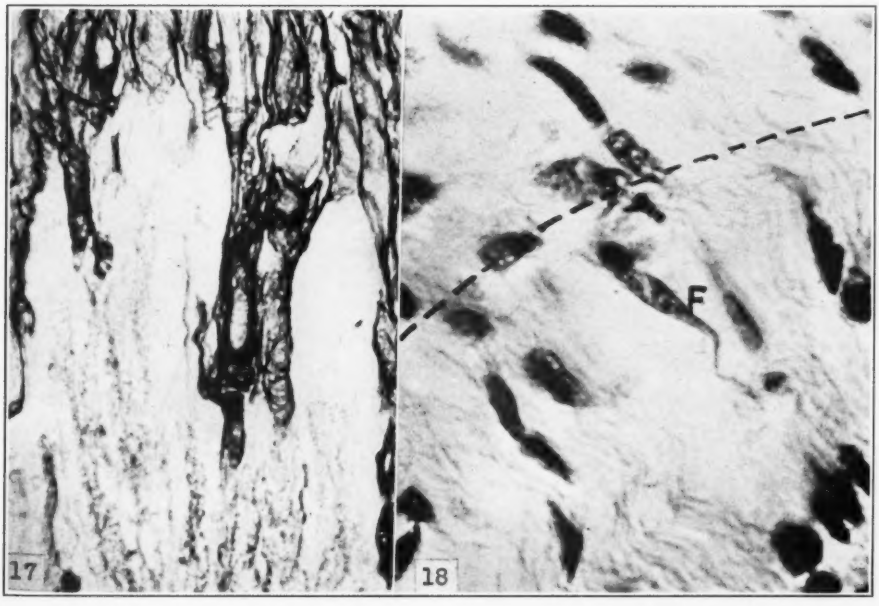
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SCHWANN CELL VERSUS FIBROBLAST AS THE ORIGIN OF THE SPECIFIC NERVE SHEATH TUMOR *

Observations Upon Normal Nerve Sheaths and Neurilemomas In Vitro

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Introduction

Since the description by Verocay [1] in 1908 of the specific nerve sheath tumor which he named neurinoma, two opposing views of its cellular origin have prevailed. One, represented by the work of Nageotte,[2, 3, 4] and of Masson,[5] is based on the study of the morphology and physiology of normal nerve sheaths under various experimental conditions. By comparing the histology of these tissues with that of the tumors, these workers feel that they have proved that the tumor originates from the sheath of Schwann. They therefore give it the name schwannoma, or peripheral glioma.

The opposing opinion, represented by the work of Mallory [6] and of Penfield,[7] emphasizes mainly the study of the extracellular components of these tumors, and rests on the assumption that collagen and reticulin can be formed only by cells of mesodermal derivation. Since the tumors contain considerable collagen and reticulin, this line of reasoning induces the conclusion that the tumors are derived from the endoneural or perineural components of the nerve sheath, and the term perineural fibroblastoma is adopted. Both schools accept Harrison's [8] demonstration in 1924 of the origin of Schwann cells from the neural crest.

Since neither side has been able to convince the other by histological methods, and since in some instances the same evidence is interpreted differently by exponents of opposing theories, an attempt has been made in this laboratory to solve the problem by

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another method. We have employed the method of tissue culture in the hope of obtaining critical evidence which the purely histological approach has failed to reveal.

The procedure has been: (1) to cultivate normal and experimental nerve sheaths *in vitro* in an indifferent medium, studying the morphology and the physiology of the outgrowing cells, thus assembling criteria by which the Schwann cell may be distinguished from its accompanying fibroblasts under conditions of uncontrolled or unorganized growth; and (2) to obtain spontaneous tumors of the nerve sheath and cultivate them under similar conditions, comparing the outgrowth with the norms established in Group 1.

Our primary aim has been to establish a concept of the general morphology and physiology of the cell types in question, when freed from normal relations and from the control exerted by the organism. Specific staining, though often an aid in diagnosis, is not wholly to be relied upon in tissue culture, nor in the study of tumors. The tumors with which we are concerned, however, are slow growing and of a considerable degree of organization; differentiation is high and cellular aberrancy at a minimum in certain areas, a fortunate circumstance for the elucidation of the problem.

The appearance and behavior of the various cell types are described and recorded as far as possible by microphotographs. By means of these the reader may compare the tumor cultures with cultures of normal and of experimental nerves of man and the rat. When dealing with a normal nerve in culture it is possible to identify the Schwann cells in the explant, as they assist at wallerian degeneration, and to observe them as they emerge in alignment from tufts of nerve fibers. Since they have a characteristic form and definite peculiarities in staining, it is possible also to recognize them when they are free and isolated in an outgrowth composed of all the constituents of the nerve sheath. It should be borne in mind that in these preparations one sees always the whole cell — never a cell dismembered by sectioning.

During the past year we have obtained and cultivated two sets of multiple tumors from two different individuals, and we now submit evidence that these are of schwannian derivation. We shall refer to these tumors as neurilemomas, the term proposed by Stout [9] in 1935 for the specific nerve sheath tumor.

Preliminary Experiments

Cultivation of Normal and Experimental Nerve Sheaths In Vitro

To prevent this paper from exceeding a convenient length, we shall give here only a brief description of the procedure and the material by means of which we have studied the morphology of the sheath cells *in vitro*, relying on microphotographs of the material to convey the essential evidence. The treatment and the culture mediums have been uniform, as far as practicable in dealing with material from different species. The Maximow hanging-drop method was used.

In the course of the investigation we have cultivated and studied *in vitro*:

(*1*) *Fetal Spinal Nerves from Man and the Rat:* The human specimens (5 months fetuses) were not particularly instructive because of the relatively undifferentiated character of the sheath cells, or because of the exuberance of the fibroblastic growth which tended to obscure other cells.

The rat material came from 20 day rat fetuses in which the spinal nerves were still unmyelinated. In many of these the sheath cells migrated and grew out freely. They were frequently discrete, though more often syncytial, with fine anastomosing filaments. With Mallory's phosphotungstic acid hematoxylin they stained deeply with a purplish cast quite distinct from the accompanying fibroblasts, which were pinkish in addition to being of a wholly different form (Figs. 1 and 2). These cultures digested fibrin with inconvenient rapidity, especially in the areas richest in Schwann cells. As a result the explant was often lost in the course of washings. The outgrowth then filled the empty space.

(*2*) *A Normal Splanchnic Nerve from a 16 Year Old Girl:* In this the growth was slow and sparse, but definitely schwannian. Syncytia and cells with long filiform branches emerged from the bundles of nerve fibers (Fig. 3). Fibroblasts were scarce or absent from this outgrowth. (The normal adult fibroblast, especially from man, grows out very slowly, as a general rule.)

(*3*) *Sciatic Nerves from Young Rats (2½ Months Old) in Simultaneous Wallerian Degeneration and Nerve Sheath Outgrowth:* It is not necessary to give a complete description of wal-

lerian degeneration here. Suffice it to say that Nageotte's [2] exact and comprehensive description of this process as it takes place *in vivo* (1922) is substantiated by our observations *in vitro*. Nageotte also placed segments of nerves in balanced salt solutions and observed the first steps in the myelin degeneration, but since the cells of Schwann did not survive in this medium the process was arrested there. In our culture medium, composed of a balanced and buffered saline solution, homogeneous plasma and tissue juice, the Schwann cells survive and become very active. As Nageotte states, they bud from the parent stalk, but within 48 hours they begin also to wander out as single units into the medium. There they may divide and the daughter cells remain attached in syncytial form. The sheath cells from the fibers of Remak are precocious; these closely resemble the embryonic form of Schwann cell referred to above. The cells from the myelinated fibers, whose cytoplasm is more abundant, nevertheless emerge later through interstices in the gelatinized Plenk-Laidlaw sheath.

Wallerian degeneration proceeds rapidly in our cultures and regeneration takes place simultaneously, after a lag of about 2 days. There is a high degree of resolution and considerable proliferation within 6 days (Figs. 4 and 5). At this time the collagenous sheaths of the large medullated fibers have the gelatinized reticulated appearance described by Masson [5] in 1932, with the longitudinal fibers staining with aniline blue (Fig. 5).

Since under normal conditions there are relatively few phagocytes present in a nerve, the transplantation of a nerve segment into culture medium allows it to pursue its degenerative course practically free from systemic interference. Under these circumstances, as has been shown, resolution of the myelin inclusions is accomplished none the less, presumably by the activity of the Schwann cells and probably by their subsequent absorption of the liquid products of this digestion. They are, as Nageotte states, slightly, but only slightly, phagocytic. These cultures have a strongly liquefactive effect on the fibrin clot in which they grow. This digestive activity suggests an explanation for the infiltrative tendency of proliferating schwannomas resulting from nerve section. The sheath cells from the Remak fibers play an important part in the migratory and proliferative activity which goes on in the cultures (Fig. 6), as do the endoneural cells. However, the

latter take no part in the resolution of the dead neurite or of its myelin sheath.

While it is impossible to exclude the possibility of amitotic division in the schwannian ribbons, it is apparent that under these conditions the usual form is mitotic (Fig. 7). Although the result of a division often suggests that the nucleus has divided longitudinally, we have seen only one longitudinal division actually take place. All the others we have observed have been transverse, with the exception of some suggestive fixed cells.

Limitations of the method have prevented us from carrying the study of regeneration *in vitro* beyond its early stages. When the nerve segment is implanted it may be teased a little with glass needles, and in successful preparations the course of degeneration may be observed. Any severe trauma destroys the Schwann cells. Even with washings, the preparations cannot be kept more than 10 days, as a rule, in their original situation. On transferal to a fresh medium the explant contracts and fibroblasts gain the upper hand, so that the culture becomes worthless for our purposes.

(*4*) *Cultivation of Schwannomas Formed In Vivo:* This material * was obtained by extirpating 1 cm. of the sciatic nerve in adult rats and by explanting portions of the proximal and the distal stumps after 7 weeks.

Here, as in the preceding section, we shall not give a detailed description of material already handled in a masterly manner by Nageotte and by Masson. The growth we used for explantation represented approximately Stages 2 and 3 in Masson's schema of the transformation of a degenerated fiber into an aneuritic bundle of regeneration.

The outgrowth of the schwannian elements was characterized by syncytial cords, single (Fig. 9), two abreast (Fig. 10), or parallel strands very close together with frequent forking but not much parallel anastomosis (Fig. 8). There were no sheets of schwannian growth such as appear in the later stages of the regeneration, and there were many clusters of discrete cells drawn out lengthwise with many single cells actively wandering. One type of cell in the outgrowth closely resembled the embryonic sheath cells or the outgrowth from the Remak fibers (referred to under heading No. 3), with somewhat hypertrophied nuclei and

* With the aid of Dr. T. P. Eberhard.

exceedingly fine, long drawn out cytoplasmic filaments (Fig. 8). The derivation of these cells, whether from myelinated or unmyelinated fiber, can only be presumed since there is no way of distinguishing them in the explant.

In all the Schwann cells both nucleus and cytoplasm were hyperchromophilic (Mallory's phosphotungstic acid hematoxylin stain). In the type of sheath cells which is assumed because of its more voluminous cytoplasm to have originated from the medullated nerve fibers, the nuclei were frequently lobated; they were somewhat hypertrophied and correspondingly endowed with chromatin, sometimes containing three nucleoli instead of the customary one or two. The cytoplasm of these cells is not always smooth and hyaline (Fig. 9). The fibroblasts had little or no share in this hypertrophic and hyperchromophilic condition, and their behavior was quite commonplace. In one tumor in which adipose tissue was present there was a considerable growth of capillary endothelium, which served for purposes of comparison.

On the subject of division it is difficult to be explicit. The appearance and disposition of the nuclei in the schwannian bands make it tempting to infer that division is frequently amitotic and longitudinal (Masson [5]). But on only one occasion have we seen a nucleus actually complete an amitotic division. We have seen a number of mitoses, particularly at the ends of ribbons. On the other hand, the number of mitoses seen among the Schwann cells is small — much less than the number among the fibroblasts although the fibroblasts are less numerous and less actively proliferating in the culture. On this ground, and on the ground that an amitotic division is difficult to detect *in vitro* (there being many nuclear constrictions proceeding from other causes, such as locomotion), one might be justified in the inference that amitosis is a frequent mode of division among the schwannian nuclei.

Among the sheath cells of the Remak fibers densely chromatic bodies, approximately spherical, are often seen which bring to mind the homogeneous bodies described in this location by Nageotte [2] in 1922. However, close observation of favorable specimens shows them to be nuclei in mitotic division. In these cultures the liquefaction of the clot was rapid and widespread, difficult to control from the standpoint of both growth and suitable histological treatment.

In none of our cultures have we observed collagen produced anew in the outgrowth by any type of cell, schwannian or mesoblastic. This is not to be wondered at since the period of usefulness for observation in our cultures is short. According to Nageotte, it requires 2 weeks, in a regenerating neuroma of the rabbit, for the primitive membrane of Schwann to reach the point where it stains red with Van Gieson's solution, and a longer time for the longitudinal collagen fibers to appear. In cultures of lymph nodes from an adult rabbit, however, McKinney [10] in 1929 found collagen fibers appearing during the 6th day. We have not so far been able to apply Laidlaw's reticulum stain successfully to cultures, probably because of their unequal thickness and because of the presence of successive layers of plasma.

In order to compare various types of syncytia, we have cultivated (*5*) *ossifying ribs of fetal rats*, in which we studied the abundant capillary outgrowths; and (*6*) *fetal skeletal muscle*, which forms long, solid multinucleated ribbons and bands.

It is evident from our material that Schwann cells are capable of separating themselves from the group and wandering off singly during the more differentiated stages of existence of the spinal nerves, as well as in the early developmental stages, as described by Harrison [8] in 1924, and by Speidel [11] in 1932. In our cultures it is more common for proliferating cells to remain in contact with one another after nuclear divisions have taken place. We have not attempted microdissection. However, many observations of living and fixed cells give the impression that they remain in organic continuity, as Nageotte's work in 1922 [2] and that of De Renyi [12] in 1929 indicate (Figs. 2, 3, 7, 8 and 9).

Résumé of Characteristics Distinguishing the Cells of Schwann from the Mesoblastic Elements of the Nerve Sheath Cultivated In Vitro

It is not our intention to state that throughout these studies we can unerringly classify any and every cell in our cultures. In tissue culture, perhaps more often than elsewhere, there are borderline cases in which certain characteristics appear to overlap. However, we do state that for the majority of the cells with which we are dealing the distinction is clear beyond any reasonable

doubt, since the Schwann cells retain a considerable degree of differentiation *in vitro*.

Outgrowing Schwann cells characteristically align themselves in formations wherein the individual cells adhere to one another at one or more points, giving the appearance of syncytia. These consist of ribbons in which the filiform cells lie tandem, or in parallel ribbons or bands with anastomosing branches. Single cells frequently dissociate themselves from the group and wander off slowly to another locality, in much the same way Speidel [11] describes in his studies of sheath cells in the developing tadpole's tail. The discrete cells are usually quite distinct from fibroblasts in form and staining. They are frequently dichotomous (Nageotte and Guyon [3]), especially at the anterior end. The syncytial structures are absolutely distinct from any syncytial arrangement adopted by fibroblasts; and though the hypertrophied ribbons from the medullated fibers may occasionally be confused by an inexperienced observer with capillary outgrowths, the texture and relative width of the cytoplasm, and the number of nucleoli, will usually dispel any doubts. The single or the syncytial structures formed by the sheath cells from the Remak fibers cannot possibly be mistaken for formations of mesoblastic cells.

Let us consider the individual Schwann cell *in vitro*: this cell is long, thin and compact, as compared with its companion fibroblasts. It has a generally bipolar form, often filiform, while the fibroblast is amorphously branching. We should mention here that the endoperineural fibroblast, like other races of fibroblasts, has a perceptible individuality. We shall not treat endothelium separately from the endoperineural fibroblast except where capillary formations are in question.

The Schwann cell has an oval nucleus which, especially in the cells from the fibers of Remak, is wider than the empty protoplasmic ribbon which contains it, thereby causing this to bulge in a characteristic manner; the diameter of the fibroblast nucleus, on the other hand, is always considerably less than the diameter of the cell. The Schwann cell nucleus in the rat and in man is poor in chromatin, and has usually one or two prominent nucleoli — only rarely three or four. Fibroblasts and endothelial cells in the same species have a variable number from two to seven, with four being the most common number. By this criterion alone a group

of Schwann cells can often be distinguished from a group of fibroblasts.

After fixatives such as Bouin's and Zenker's, the cytoplasm of the Schwann cell is smooth in texture as compared with the granular or fibrillar fibroblast; in the embryonic material and in the Remak sheath especially it has a hyaline appearance. With Masson's trichrome stain, also with Mallory's phosphotungstic acid hematoxylin, the Schwann cell shows certain clear and definite peculiarities in coloring. Its cytoplasm takes a deep clear red in the former, and a bluish purple in the latter stain. Nageotte likewise reports a distinctive picture for the Schwann cell by the use of hemalum after treatment with alcohol and nitric acid. In his figures of Schwann cells of the unmyelinated neurites of the cornea, and of the fibers of Remak, Nageotte gives illustrations that can be duplicated to the minutest essential detail in our cultures of nerves and in the cultures of the tumors under discussion.

The Spontaneous Tumors

Case 1: The patient was a male neurologist (S.P. 69049) aged 68 years, with tumors in the left posterior tibial nerve in the popliteal space, in the right ulnar nerve near the head of the ulna, and in a branch of the anterior crural nerve beneath the rectus femoris muscle in the left thigh. These tumors had been present for several years and had recently occasioned tingling in the left foot and right hand. All of them lay within the sheaths of the respective nerves with the nerve fibers spread out over their surfaces. They were encapsulated and were removed by pushing the nerve fibers aside and enucleating them. They were all essentially the same in gross appearance, without any gross cyst formation. The cut surfaces were a mottled yellowish gray. The largest tumor came from the posterior tibial nerve and measured 25 by 20 by 22 mm. The others were only half as large. There was no evidence of von Recklinghausen's disease.

All three tumors show both A and B type tissue (Antoni [13]) with marked organoid differentiation, especially in Tumor 2 from the ulnar nerve, and in Tumor 3 from the anterior crural nerve. In Tumor 1 there are blood vessels with thick collagen sheaths, but in Tumors 2 and 3 the blood vessel walls are little if any thickened. Sections impregnated by Cajal's method show neurites

scattered at intervals throughout all three tumors. They are delicate and non-myelinated, and course at haphazard throughout both the A and the B tissues. All three tumors were considered characteristic neurilemomas.

CASE 2: The patient was a negress (S.P. 69535) 28 years old, with three tumors. The largest tumor measured 12 by 7.5 by 3.5 cm. and lay within the sheath of the right sciatic nerve in the midthigh, where its presence had been noticed for 6 years. The other tumors were of shorter duration. One tumor 4 cm. in diameter lay within the sheath of the right tibial nerve in the popliteal space, and the other was attached to a branch of the peroneal nerve just posterior to the right medial malleolus. The tumor attached to the tibial nerve caused some pain which radiated down to the great toe and the heel. The others were symptomless. There was no evidence of von Recklinghausen's disease. All of these tumors were excised by enucleation, sparing the nerves as much as possible. Following the operation there were some disturbances of sensation.

The tumor from the sciatic nerve was encapsulated and largely cystic, with spaces filled with gelatinous and sometimes blood-tinged fluid. The other tumors were less cystic but were encapsulated and made up of both soft and firm, yellowish gray semitranslucent tissue.

All of the tumors are composed of both A and B types of tissue without much organoid differentiation but with collagen and reticulum fibers in both types and long tapering cells sometimes arranged in tandem formation with two or more nuclei. Cajal impregnations show delicate, non-myelinated neurites coursing in all directions in both the A and B type tissues in the two smaller tumors, but none is found in the largest tumor from the sciatic nerve. All of the tumors have blood vessels surrounded by thick collagen sheaths. They were all accepted as neurilemomas.

THE TUMOR CELLS IN VITRO

When the tumors are being cut up for examination, and especially after the explants have been placed on coverslips, it is usually possible to distinguish between the A type and the B type explants. The A type has a whitish, shiny, almost translucent appearance, while the B type is blotchy, opaque, and often yellow-

ish. The fasciculated form of the A type often may be discerned at the edges of the explant.

Type A Tissue: All the distinguishing characteristics of the Schwann cell *in vitro*, evident in mixed cultures from normal peripheral nerves and from experimental schwannomas, may readily be seen in the outgrowth from the A type tumor tissue, and none of the distinguishing marks of the fibroblast are found (Figs. 11, 13). The tissue has a pronounced liquefactive effect (very difficult to control) upon the plasma clot in which it is grown. It has a tendency to adhere to surfaces, healing over cut edges, and in many instances it grows out in the form of sheets or cords (Fig. 14). These latter probably emerge from the palisaded nodules. The A type of tissue does not seem to undergo degeneration or dedifferentiation *in vitro*. At the end of 12 weeks cultivation it still grows out in the original form even though parts of the explant may have accumulated fatty degeneration granules. Sometimes sheets of interlacing cells are formed. These are always inconveniently liquefactive.

In the outgrowth from normal sciatic nerves undergoing wallerian degeneration *in vitro* there are present a great many sheath cells from the fibers of Remak. These can be identified by appearance and behavior while still situated in the nerve bundle, or when emerging from it, or at some distance away in the outgrowth (Figs. 4 and 6). Their hyaline, threadlike cytoplasm is distinctive, as is also their small size. Their anastomosing branches which rejoin the parent stalk (Fig. 6) are instantly to be recognized in Nageotte's figures of such cells,[2] which have been deprived of their nerve fibers. They have differentiated very little beyond the embryonic type of Schwann cell (Figs. 1 and 2).

The outgrowth from the A type areas of the tumors is composed very largely of such cells, slightly hypertrophied and of slightly more than normal variation in size. They are the most stable type of cell in the tumors. The picture once seen is unmistakable (Figs. 11–13), and appears in culture after culture. This is the morphological norm in cultures of the A type areas, overshadowing the sheets and cords, which probably emerge from palisaded nodules (Fig. 14).

It is not surprising that this should be so when one considers the large numbers of Remak's fibers which are carried by the

peripheral nerves. Ranson *et al.*[14] have made careful counts of the myelinated and unmyelinated nerve fibers in a number of the spinal nerves in man, and find widely varying ratios between them but always a substantial number of Remak's fibers. For instance, the superficial branch of the ulnar nerve gives 2.12 unmyelinated to 1 myelinated nerve fiber; the deep branch 0.69 unmyelinated to 1 myelinated; in the lateral cutaneous nerve of the thigh, 3.85 unmyelinated to 1 myelinated. It will be seen, therefore, that although the bulk of the peripheral nerve appears to be taken up by the myelinated fibers, this is a false impression due to the relatively large size of the individual fibers. It is therefore not difficult to conceive, on grounds of opportunity, that the sheath cells of unmyelinated fibers might afford an origin for the A type of tumor tissue.

The Palisaded Nodules: A second type of growth is sometimes obtained from the hyaline explants, probably from the palisaded nodules which they contain. This is more solid than the usual A type of outgrowth, and resembles the sheets and cords formed by epithelium when it is growing through a clot, as these cells are (Fig. 14). After 12 weeks cultivation, in which it produced only this type of outgrowth, one of these cultures began to produce large numbers of cells characteristic of the B type of explant, which is described below.

Type B Tissue: Cultures of the B type, or reticulated tumor areas, produce cells that at first appear to be quite different from those of the A type. They liquefy their medium so rapidly that in the vicinity of the explant, where they are present in greatest concentration, they are often found in the retracted form induced by lack of solid support, and bear a superficial resemblance to mesothelial wandering cells (Fig. 17). They do not, however, stain brilliantly with neutral red intravitally, as these latter cells do; a few granules in them stain a pale orange, and only occasionally are there bright red inclusions. Furthermore, the nucleus, when fixed and stained, is entirely unlike that of the macrophage or lymphocyte (Fig. 16).

Where the clot is dense enough for them to exist in the extended state the B cells are elongated, knobby and branching, and much more irregular in form than the A type. Because the nucleus tends to be less vesicular, it is thinner and more elongate, and the

ratio of cytoplasmic to nuclear diameter is greater than in the cells of the A type; hence the nucleus does not bulge conspicuously from the band. The average number of nucleoli is less than two. The nucleus is often lobated or constricted, suggesting amitotic division, or, where the cytoplasm is constricted in the same area, suggesting locomotion by constriction bands as described by Speidel. The cytoplasm is not as hyaline as in the A type but tends to be opaque. These cells may form syncytial bands in tandem (Fig. 15), but often are united into an amorphous appearing structure reminiscent of the reticulated B tissue from which they emerge (Fig. 16).

If a small strand of cotton or a fine rod of spun glass is introduced into the medium in which these cells are wandering, they tend to accumulate on it, losing the opacity which characterizes them in the actively wandering state and often sending out tendrils along and around the rod of support (Fig. 18). Sometimes a pocket of liquefied plasma can be seen between the cell and the glass rod.

These cells were the first to make their appearance in the cultures. At the end of 12 weeks cultivation they were still present in scarcely modified form. They show a generic similarity to the cells of the A type but maintain also these constant differences. One is obliged to call them Schwann cells. They are not fibroblasts, endothelium or macrophages.

Although the B type areas of the explants gradually accumulate fatty granules and vacuoles *in vitro*, the B type of cell for a long time continues to migrate from them, and once free in the medium is very active. Of the two cell types it has the shorter lag period and the greater capacity for digesting fibrin. It does not die out and there is no clear evidence that under tissue culture conditions it metamorphoses into the A type, unless it be sometimes into sheets or cords. In Figure 17, which shows clumps of B type cells in an extreme liquefactive state, such that the medium is wholly fluid, a few cells may be seen in a more normal form, extended against the coverslip which affords them support. The whole picture of the B type cell is not primarily that of a degenerating cell but of a cell that lacks osmotic equilibrium; of one that is somewhat plasmolyzed as the result of excessive secretion of digestive ferments.

The stellate, retiform shape of the B type cells in the plasma clot is similar to that of the normal Schwann cell on its medullated fiber with its extensions into and through the myelin. *In vitro* these cells form syncytial structures with prolongations anastomosing in a network; essentially a reproduction of the Büngner bundles described by Nageotte and by Masson in experimental schwannomas. Only the directive forces for compressing them or of pulling them out lengthwise are (of necessity) lacking *in vitro*. The Büngner bundles appear to be derived from the sheaths of the medullated nerves.

The solitary cells or tandem arrangements in the tumor cultures are similar in appearance to cells seen on or emerging from medullated fibers during wallerian degeneration *in vitro* (Figs. 3 and 5). They are not incompatible with Nageotte's illustrations of wallerian degeneration *in vivo*, although this stage precedes the tumor formation in the stump. They are similar to Masson's figures of young schwannian bands invading the connective tissue in experimental schwannomas. In our cultures of experimental schwannomas this type appears also (Fig. 9).

Endothelial Elements: Endothelial elements in the cultures are even less conspicuous than in the sections of the tumors. There were at the beginning of cultivation a number of true wandering cells of lymphocytic nature, as in practically all tumors, but these were quickly lost. There was no capillary formation *in vitro*.

Cell Division: In the matter of cell division in these tumor cultures it is difficult to be explicit. In the whole course of our observations we have seen but two mitoses, both in outgrowths from the palisaded areas. Although it is true that the rate of outgrowth in the A type is slow, still it seems incredible that more mitoses should not have come to light if mitosis were the sole method of division. In the B type particularly, whose outgrowth or outwandering is not slow, no mitoses have been seen, but many nuclear constrictions have been present. In both types (A and B) unequal nuclear divisions are common. It seems unlikely that in the course of 12 weeks cultivation and many passages there should be no cell division; one is therefore impelled to regard amitosis as the probable method.

Inclusion of Normal Tissue with Tumor: The third tumor from Case 2 had infiltrated a considerable portion of the endoneurium

of a branch of the right peroneal nerve. Consequently when it was removed it was necessary to take a good deal of the nerve with it. In several cultures of this tumor we had areas of normal nerve, and from these normal Schwann cells and characteristic endoneural fibroblasts grew out.

Discussion

It should be emphasized that in our cultures of these two sets of neurilemomas no typical fibroblasts have appeared in the outgrowth, and (except in two cultures from a tumor which contained strands of normal nerve) nothing even remotely similar to the endoperineural fibroblasts which are common in our cultures of normal nerves in wallerian degeneration has been observed. Yet the tumors are quite well supplied with collagen in the B type areas, and reticulin or collagen in the A type areas.

It is evident that the neoplastic elements in this material are represented by the two types of Schwann cells emerging from the A and B type areas of the tumors, and by the epithelioid growths emerging from the palisaded nodules.

That Schwann cells can and do form collagen and reticulin seems a reasonable inference from our observations, since these tumors of peripheral nerves containing neurites and solid homogeneous bands of tumor cells are well supplied with fibers that stain with aniline blue and with silver. Characteristic tumor cells, which appear to be schwannian, invariably grow out from the explants, but fibroblasts do not. Careful and repeated examination of the tumor sections has not disclosed the presence of a stroma, unless the sharply circumscribed collars around blood vessels could be given that name. Consequently the conclusion seems strongly indicated that the collagen and reticulin which characterize the A and B type areas are formed under the influence of the A and B type tissues — which our evidence classifies as schwannian.

The presence of two rather distinct and stable cell types in the outgrowth from the tumors is especially interesting in the light of the classical morphology of the neurilemoma — *i.e.* its tendency to be divided into A and B type areas, so-called. Observations on the physiology of the two cell types *in vitro* do much to explain the differences in morphology between the A and the B type areas,

which are regarded, we believe rightly, to have the same cellular origin.

The B type of tissue was thought by Antoni to be a degenerative phase of the A type. Yet, cultivated *in vitro*, these cells do not die; on the contrary they are highly active metabolically and they persist, constantly migrating out from the explant, through many passages. The life of these cultures was never terminated spontaneously during the 3 months through which they were kept growing. It may be of significance that in our two sets of three multiple tumors the largest tumors have contained the largest proportion of B type tissue. *In vitro* this type maintains its individuality, thus presenting us with the problem of a tumor in which there appear to coexist at least two types of Schwann cells.

We have speculated inconclusively whether this situation could be the result of the presence of two types of Schwann cells in the nerve of origin. Certainly two types of nerve fiber, each with its investiture, are there. Speidel's observations indicate that while there is no specificity of Schwann cells in the early phases of fiber development, and while a cell from a non-myelinated fiber may migrate at any time to a myelinated fiber, the relation of cell to myelinated fiber, once established, is quite a stable one. Possibly, therefore, the subsequent differentiation of the Schwann cell is not altogether reversible and we may be dealing with two types of sheath cells which have a tendency to remain distinct. In cultures of normal nerves we have often found it possible to distinguish between the two types. There is a striking resemblance between the A type tumor outgrowth and the outgrowth from the Remak fibers; and except in its extremely liquefactive phases the B type tumor outgrowth bears considerable resemblance to that from the medullated fibers. If our hypothesis should be verified, we should have to believe that the palisaded nodules arise from the sheath cells of medullated nerves, since their outgrowth has been seen *in vitro* to metamorphose into the B type. It may be of interest in this connection that the Wagner-Meissner corpuscles are found at terminal branches of myelinated nerve fibers.

In 1916 Ingebrigtsen,[15] studying the phenomena of nerve degeneration, cultivated sections of sciatic nerves from rabbits, which had been allowed to degenerate *in vivo* for 5 days or more. In the schwannian outgrowth which he obtained he made morpho-

logical distinctions between the outgrowths from nerves in the earlier stages of degeneration, which tended toward a "syncytial network," and straight outgrowths composed of parallel rows of cells similar to the bands of von Büngner, from the later stages of degeneration. His figures suggest that in his cultures it would be possible to distinguish the cells from the medullated fibers from those of the fibers of Remak, and that the cells from the medullated fibers form the bulk of the later outgrowth. His figures also bear a generic resemblance to our two types of tumor cells.

An alternative hypothesis to that of double schwannian origin just outlined, would regard the differences observed among the normal and experimental Schwann cells as non-specific; *i.e.* as relating merely to the cells' original dimensions or degree of hypertrophy. The different tumor areas and types of tumor outgrowth would then represent degrees of tumorous modification of the original Schwann cell, with the A type as the least modified, and the cystic B type as the most modified. There is evidence to support this theory also in both the tumor sections and the cultures of normal and tumor cells. But we are not able at this time to offer conclusive observations on either side. It is difficult to fit the palisaded nodule satisfactorily into either picture.

The progressive degeneration, jellification and eventual liquefaction of the collagen and reticulin in the B type areas of tumors is not of itself remarkable if the behavior of the B type cells *in vitro* be taken into consideration. It will be remembered that they have a marked proclivity to digest the fibrin clot into which they have grown or migrated, and thus to surround themselves by liquid areas from which their protoplasm is retracted, a striking equivalent of microcystic degeneration. When one considers the intimate nutritive relationship which is normally maintained between the nerve fiber and the Schwann cell, and the latter's influence on both the formation of myelin (Speidel [11]), and on its resorption in wallerian degeneration (Nageotte [2]), one may conclude that the normal Schwann cell of the medullated nerve is a particularly active producer of digestive catalysts. After injury to the nerve, or sectioning of it, the Schwann cells not only become hypertrophied and proliferate, but often infiltrate their surroundings as well. A tumor cell, aberrant in respect to its reproductive function, and deprived of its normal relation with the nerve fiber,

might be expected to suffer an exaggeration of its normal digestive function as a result of its general disequilibration.

Hyperactivity of the liquefactive function, as in newly emerged B type cells from cystic areas, works against the morphological expression of the cells' differentiation, even *in vitro*, by the liquefaction of the fibrin adjacent to the cell and the coincident destruction of the solid support for finely branching processes (which characterize the normal Schwann cell *in situ*). These, therefore, are retracted and appear as the knobby branches typical of this type of tumor cell *in vitro*. Experimentally this condition may be overcome to some extent by providing the cells with an exceedingly thick clot which they cannot easily liquefy. In the outgrowth of B type cells from sclerosed portions of tumors, the morphological differentiation appears unhindered since it is not digestion but collagen formation which is the aberrant function here. These cells are often solitary and are provided with numerous finely branching processes. It is this condition which predominates in cultures of tumors generally classified as neurofibromas.

By the train of reasoning outlined above it is possible to bring into harmony the phenomena of sclerosis and of Antoni's B type of tissue, or myxoid metamorphosis, as being due to the unbridled exercise of different normal proclivities by disequilibrated sheath cells. The choice and the timing of the aberrancy among these respective functions might well be dependent on local or systemic conditions varying within the tumor and from tumor to tumor. Where the cell engages in either sclerosis or liquefaction in great excess, it is inevitable that it should destroy itself, as uncontrollably liquefactive cells do *in vitro*.

Summary

We have offered evidence, obtained by means of tissue culture, that the specific nerve sheath tumor or neurilemoma is of schwannian origin. This evidence is based on morphological and physiological similarities between the schwannian outgrowth from normal and experimental nerves and the outgrowth from spontaneous tumors. It is inferred that Schwann cells can and do condition the formation of collagen without the intervention of fibroblasts, since these tumors, whose outgrowth is wholly schwannian, contain considerable collagen.

By the study of the tumor cells' behavior *in vitro* we are able to offer an explanation of the so-called cystic degeneration in these tumors and to harmonize it with the sclerosis seen in neurofibromas.

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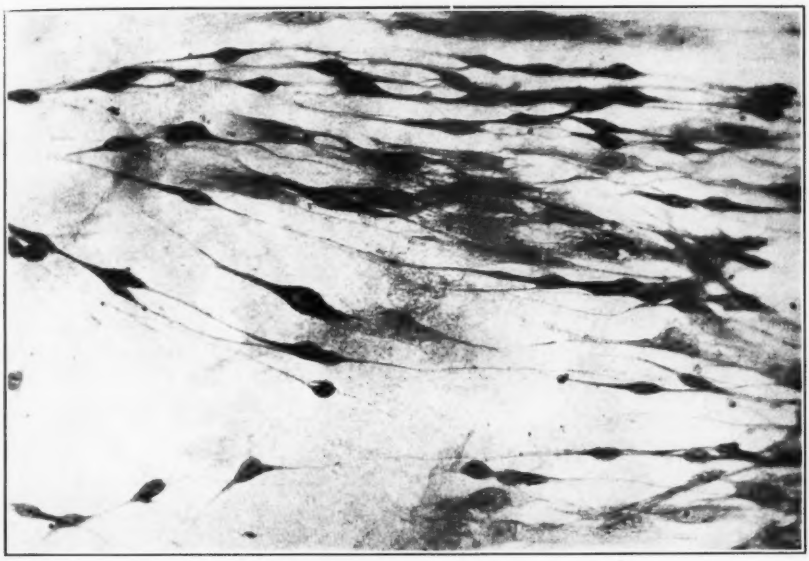
DESCRIPTION OF PLATES

Plate 10

Fig. 1. Schwann syncytium from the spinal nerve of a 20 day rat fetus. One fibroblast is seen at the right. Tissue culture 1 week *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.

Fig. 2. Schwann syncytia and fibroblasts from the same culture as in Figure 1.



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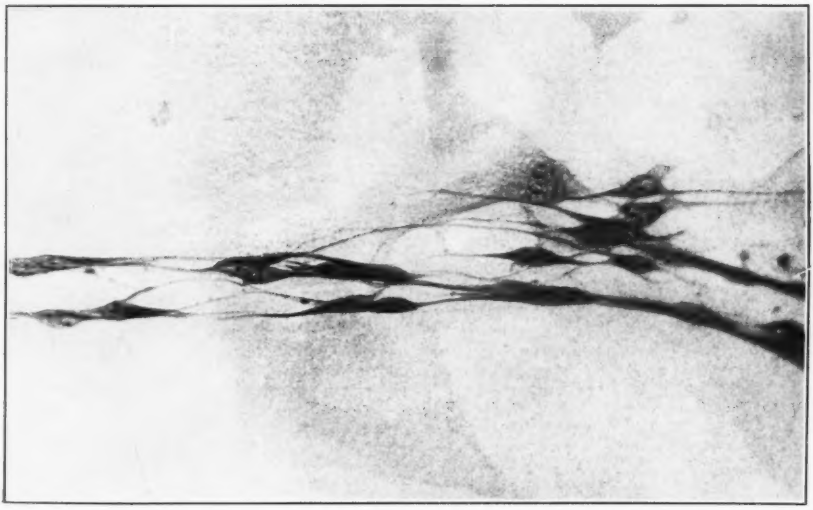

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Murray and Stout Schwann Cell Versus Fibroblast

PLATE 11

FIG. 3. Schwann cells from the splanchnic nerve of a 16 year old girl. Tissue culture 10 days *in vitro*. Toluidine blue stain.

FIG. 4. Wallerian degeneration *in vitro*. Note the Remak fibers. Sciatic nerve from a 2½ months old rat. Tissue culture 8 days *in vitro*. Phosphotungstic acid hematoxylin stain.

4

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Murray and Stout

Schwann Cell Versus Fibroblast

PLATE 12

FIG. 5. Wallerian degeneration *in vitro*. Schwann cells of medullated fibers. Sciatic nerve from a 2½ months old rat. Tissue culture 6 days *in vitro*. Zenker's fixation, Masson's fuchsin-ponceau, aniline blue stain.

FIG. 6. Cells from Remak's fibers; endoperineural fibroblasts in the background. Same culture as shown in Figure 5.

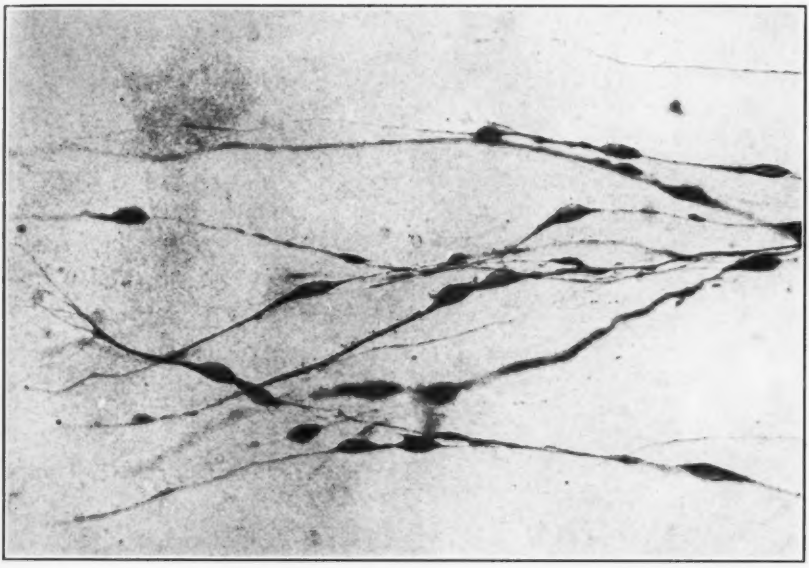
5

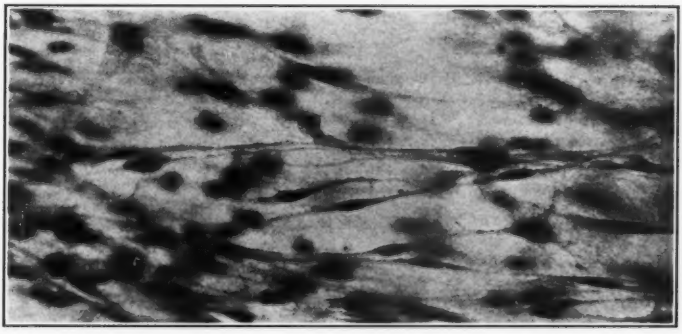
6

PLATE 13

FIG. 7. Early stage in palisading. Outgrowth from a sciatic nerve from a rat. Tissue culture 9 days *in vitro*. Delafield's hematoxylin stain.

FIG. 8. Schwann syncytia from a proximal amputation neuroma (7 weeks) of a sciatic nerve from a rat. Tissue culture 5 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.





Murray and Stout

Schwann Cell Versus Fibroblast

PLATE 14

FIG. 9. Schwann cells from a distal amputation neuroma. Same procedure as in Figure 8.

FIG. 10. Double schwannian band from a distal amputation neuroma. Same procedure as in Figure 8.

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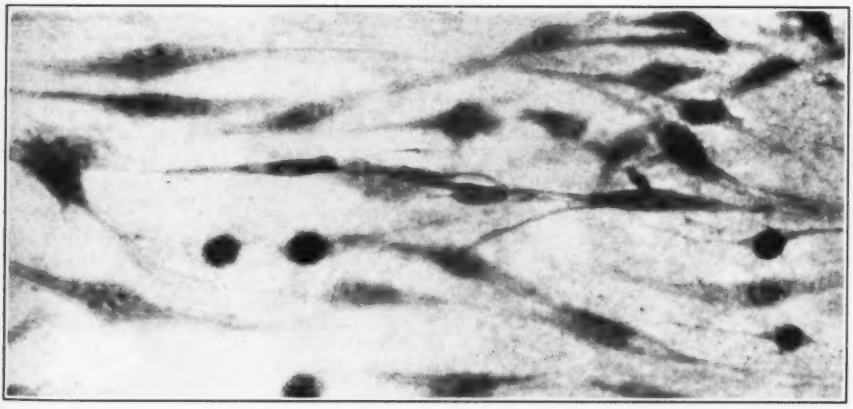

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Schwann Cell Versus Fibroblast

PLATE 15

FIG. 11. Outgrowth from A type area of neurilemoma, Case 1. Tissue culture 19 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 12. Another portion of the same culture shown in Figure 11.

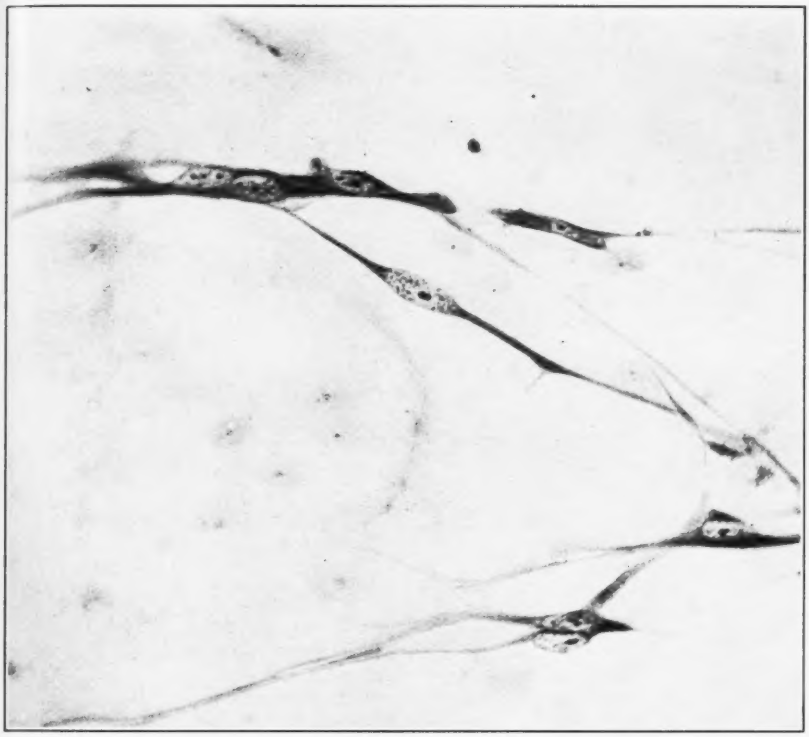

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PLATE 16

FIG. 13. Outgrowth from A type area of neurilemoma from Case 1. Tissue culture 13 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 14. Outgrowth from a palisaded nodule, Case 1. Tissue culture 13 days *in vitro*. Bouin's fixation, phosphotungstic acid hematoxylin stain.

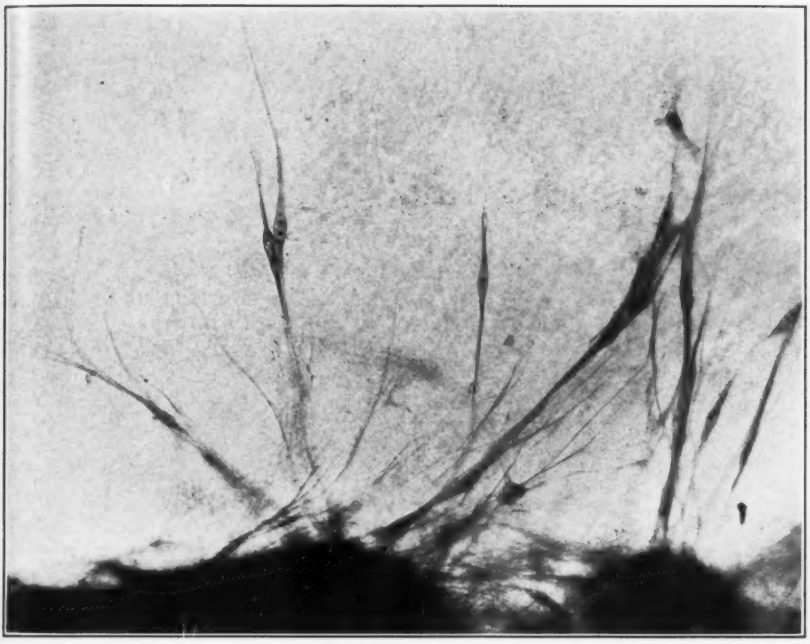

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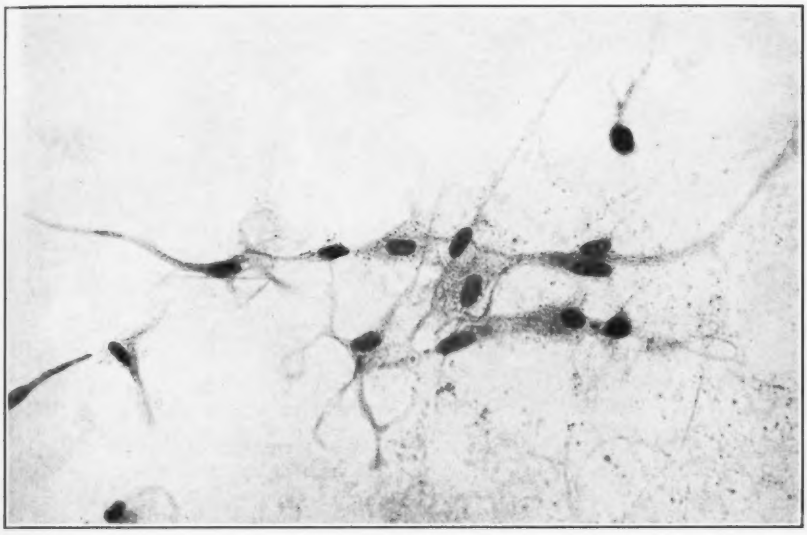
PLATE 17

FIG. 15. Outgrowth from B type area of neurilemoma, Case 1. Tissue culture 8 days *in vitro*. Bouin's fixation, phosphotungstic acid hematoxylin stain.

FIG. 16. From B type area, Case 1. Zenker's fixation, phosphotungstic acid hematoxylin stain.



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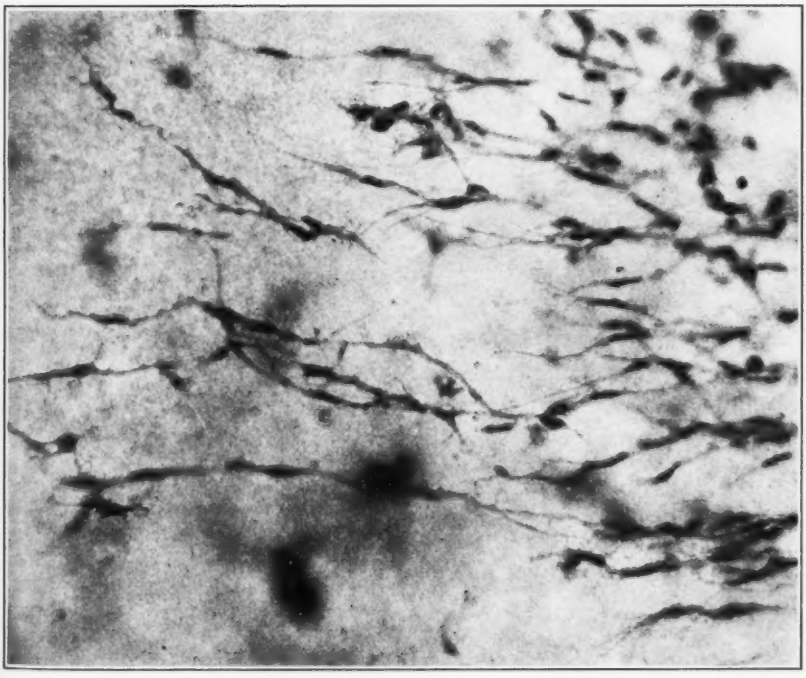

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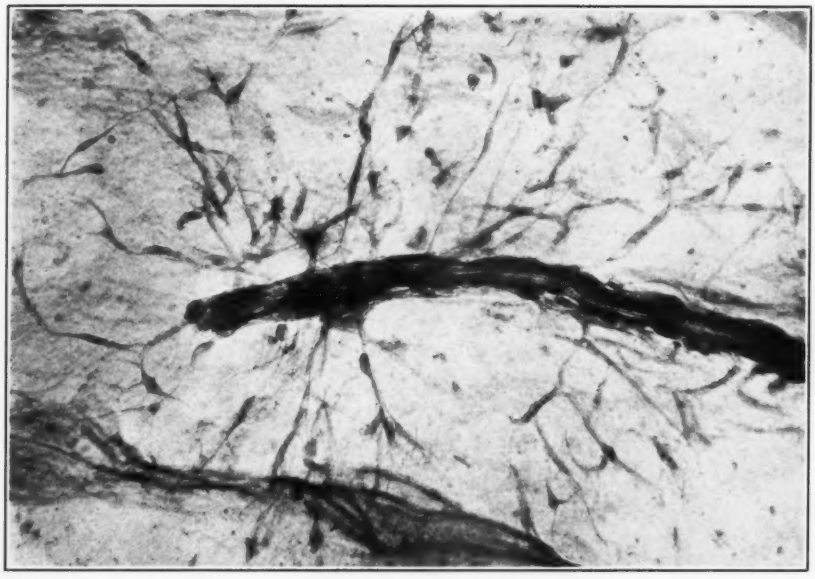
Murray and Stout Schwann Cell Versus Fibroblast

PLATE 18

FIG. 17. From B type area, Case 1. Extreme liquefaction. Tissue culture 8 days *in vitro*. Bouin's fixation, Delafield's hematoxylin stain.

FIG. 18. B type cells on a cotton fiber, Case 2. Tissue culture 7 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.



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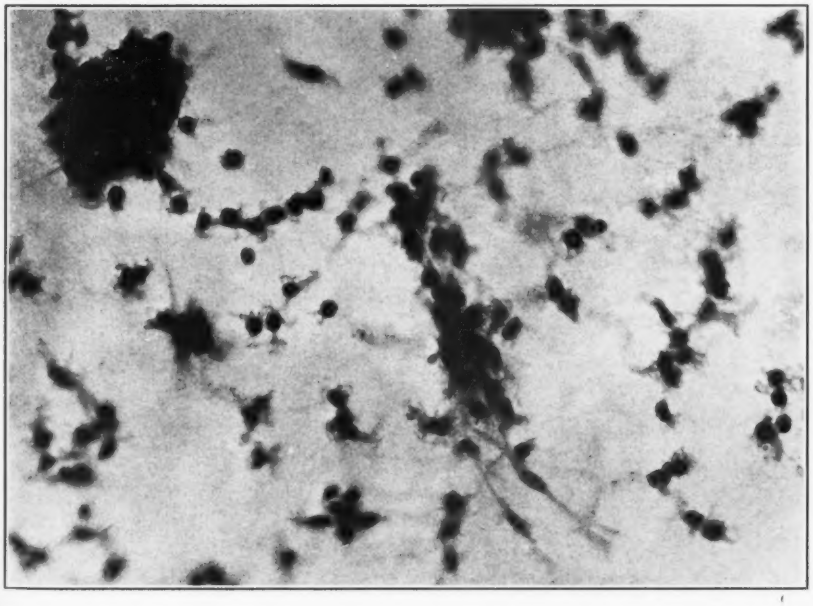

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Schwann Cell Versus Fibroblast

WERNICKE'S DISEASE *

Identity of Lesions Produced Experimentally by B_1 Avitaminosis in Pigeons with Hemorrhagic Polioencephalitis Occurring in Chronic Alcoholism in Man

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In a series of experiments reported in 1938 by Alexander, Pijoan, Myerson and Keane,[1] Wernicke's disease was produced in pigeons deficient in vitamin B_1 by adding ample supplies of other vitamins to their diet. The results may be summarized briefly as follows:

1. Wernicke's hemorrhagic polioencephalitis can be produced with significant regularity in pigeons deficient in vitamin B_1 as a complication of beriberi if large supplies of other vitamins (A, B_2, C and D) are fed.

2. If the pigeons are kept on an entirely vitamin-free diet the resulting beriberi will only rarely be complicated by lesions of the Wernicke type.

3. Wernicke's disease cannot be produced in pigeons receiving crystalline vitamin B_1 (thiamin), although they may be deprived of all other vitamins, or of any one other vitamin for a period of over 6 months.

4. These observations suggest that vitamin B_1 possesses anti-angiodegenerative properties, in addition to antineuritic properties, and that smaller amounts of vitamin B_1 are sufficient to act as an anti-angiodegenerative agent than are necessary for antineuritic action.

5. The administration of high doses of vitamins A, B_2, C or D in vitamin B_1 deficiency probably raises the vitamin B_1 requirement of the tissues, and angiodegeneration manifests itself soon after the onset of the neuronal degeneration.

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In 1939 Campbell and Biggart [2] reported 8 cases, and Ecker and Woltman [3] 1 case in which Wernicke's disease occurred in states of vitamin deficiency in the complete absence of alcoholism. Both of these reports refer to our experimental work.[1]

Prior to our experiments, Prickett [4] observed in rats fed a diet free from vitamin B_1 (heat labile fraction), but containing an adequate amount of the heat stable fraction (G), disseminated foci of petechial hemorrhage or marked congestion, usually bilaterally symmetrical, in the pons, medulla oblongata and cerebellum, in which there was cellular damage and in some instances reactive proliferation of glia, particularly of oligodendroglia and scavenger cells, and later of astrocytes. Prickett found similar lesions in a few pigeons in the pons, medulla oblongata, cerebellum, optic lobes and cerebrum. However, he did not investigate the underlying vascular disease and its identity with Wernicke's disease in man. Three years earlier Pappenheimer and Goettsch [5] described foci of edema, necrosis and petechial hemorrhage limited to the cerebellum in chicks fed a diet presumably deficient in vitamin E, although the authors consider that vitamin B_1 may also have been inadequately supplied; the supply of vitamin B_2 was ample. These authors mention vascular changes but do not discuss their possible relation to Wernicke's disease in man.

The validity of our conclusions depends on the question whether or not the lesions observed in our experiments on pigeons are identical with those occurring in hemorrhagic polioencephalitis in chronic alcoholism in man. In order to settle this point we have compared 16 cases of Wernicke's disease in man with the same number of experimentally produced cases of Wernicke's disease in the pigeon. Twelve of the human cases were due to chronic alcoholism. Two were seen in scurvy, 1 in a case of pernicious anemia, and 1 in a cachectic individual suffering from a depressive psychosis. The pigeons from our experimental series which were used for this comparative study were all deficient in vitamin B_1 and received high doses of riboflavin phosphate or nicotinic acid, or of vitamins A and D with or without addition of vitamin C.

Our contention that Wernicke's disease in man and the syndrome produced by us in pigeons by a regimen of disproportionate supply of other vitamins in vitamin B_1 deficiency are identical is

based on the gross appearance and on the topographical and histological analogy of the lesions.*

Wernicke's disease can be defined pathologically as a syndrome characterized by small foci with degeneration and varicose deformity of vessels, and subacute necrosis of the parenchyma, found predominantly around the ventricular system. Small petechial hemorrhages may frequently but not always be present throughout the lesions. Wernicke himself named the disease "polio-encephalitis hemorrhagica superior et inferior." [6, 7]

The lesions are usually symmetrical. The regions most constantly involved are the paramedian and paraventricular nuclei of the thalamus and hypothalamus, the mammillary bodies, the periaqueductal region of the midbrain, the nuclei triangularis and Bechterew of the vestibular nerve, and the dorsal nuclei of the vagus (Text-Fig. 1). It is reasonable to assume that the disease progresses from the oral toward the caudal regions.[8]

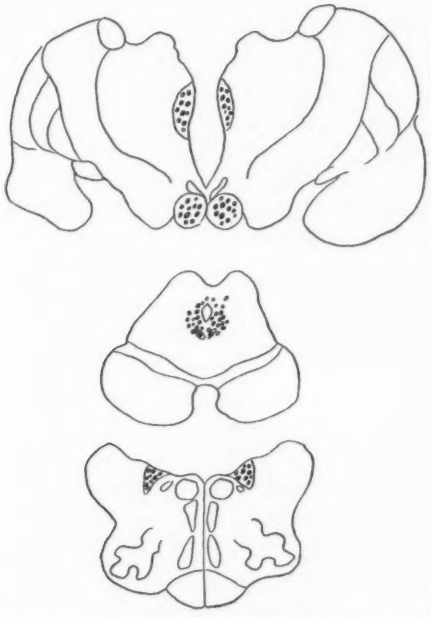
The lesions of Wernicke's disease can be recognized in gross, particularly if they are associated with petechial hemorrhages, but hemorrhages are not present in all instances. With experience one may recognize the blue-gray discoloration of the periventricular tissue as suggestive of Wernicke's disease in the absence of grossly visible hemorrhages.

Figures 1, 2 and 3 show fresh unstained slices through the brain in a case of Wernicke's disease in chronic alcoholism in man in which the petechial hemorrhages and the subacute necrosis of the tissue within the lesions are equally striking. Figure 1 particularly shows the lesions in the paramedian nuclei of both thalami outlined by discoloration and pin-point hemorrhages as distinctly as in a schematic anatomical illustration. The mammillary bodies are similarly affected. Figure 2 shows lesions ventral to the Sylvian aqueduct and in the substantia nigra on each side. Figure 3 shows the lesions in both dorsal nuclei of the vagus, on the right more than on the left.

Such gross views can be obtained by slicing the fresh brain transversely. Care should be taken to cut one of the slices through the mammillary bodies. An instructive view can also be

* Our findings were recently confirmed by Zimmerman for the pigeon and other laboratory animals. (Zimmerman, H. M. The pathology of the nervous system in vitamin deficiencies. *Yale J. Biol. & Med.*, 1939, 12, 23–28.)

obtained by making one median sagittal cut which separates the corpus callosum and opens the third ventricle. By inspection of the lateral wall of the third ventricle the hemorrhages, as well as the blue-gray areas of subacute necrosis, can be seen shining through the ependymal covering of the third ventricle. Such a section is illustrated in Figure 4, from a case of scurvy in a non-



TEXT-FIG. 1. A schematic diagram showing the distribution of the lesions in Wernicke's disease. Note the lesions in the paramedian nuclei of the thalamus, in the mammillary bodies, in the periaqueductal region of the midbrain, and in the dorsal (pneumogastric) nuclei of the vagus.

alcoholic patient. The lesions can be seen there in the paramedian nuclei of the thalamus and hypothalamus dorsal and caudal to the mammillary body.

The gross appearance of the lesions of Wernicke's disease in the pigeon is similar to that of the lesions of Wernicke's disease in man. The gross lesions in the pigeon's brain, similar to those in the human brain, consist of pin-point hemorrhages within areas

of bluish discoloration of the tissue due to subacute necrosis. Figures 5 and 6 show median sagittal slices of fresh brains of pigeons with Wernicke's disease produced experimentally. Figure 5 (Pigeon 3, Group IV_{2B}) shows gross lesions in the paramedian and paraventricular nuclei of the thalamus and hypothalamus and the region of the eye muscles. Figure 6 (Pigeon 2, Group IV_{2B}) shows gross lesions predominantly in the region of the eye muscles, but also in the paramedian nuclei. Both these pigeons had been fed a diet deficient in vitamin B_1, but had received large doses of riboflavin extracted from yeast and esterified with phosphoric acid (0.02 mg. daily) and vitamin C (13 mg. daily).

The histological appearance of Wernicke's lesions, as studied with ordinary stains, particularly the hematoxylin and eosin stain, is frequently less striking than the gross appearance, that is, the lesions stand out less conspicuously at low magnification. However, the lesions stand out strikingly if studied with histological methods adapted to demonstrate the vascular tree, the best of which for this purpose is the benzidine stain (Lepehne-Pickworth [9]).

In specimens stained by Perdrau's method, or in specimens stained according to the technic of Achucárro-Klarfeld, the lesions of Wernicke's disease stand out quite conspicuously also, but such specimens give a fallacious appearance of increase in the number of blood vessels in the lesions, although in reality only the endothelial and the adventitial elements have proliferated. This is due to the fact that specimens stained by these two methods fail to show the finer ramifications of the normal vascular tree in normal tissue with adequate contrast, and thereby give the false impression of increased vascularity in the lesions as compared with normal tissue. Many authors have been misled by this fallacious appearance. In reality, however, there is no increase in the number of blood vessels in these lesions, only irregular dilatation and an increase in the endothelial and adventitial elements. The number of blood vessels within the lesions is either unchanged or even diminished because of occlusion and obliteration of capillary and precapillary tributaries of such larger vessels as are destroyed by hemorrhages (Figs. 7, 12 and 16).

The benzidine stain not only gives a more striking and truthful

picture of the lesions of the Wernicke type by disclosing the characteristic varicose deformities of their vascular bed and contrasting them vividly with the regular slender outline of the vessels within the neighboring normal tissue (Campbell, Alexander and Putnam [10]), but in addition is likely to disclose the lesions in the earliest stage when the only demonstrable change present is this characteristic dilatation and varicose deformity of the vascular bed. This dilatation and varicose deformity in lesions of the Wernicke type involve vessels of all orders, that is, the capillaries, the precapillaries and the larger intracerebral vessels of the third and the fourth order within the lesions.

After comparing benzidine stained preparations in our series of cases with specimens stained according to the usual methods, I have come to the conclusion that angiodegeneration with varicose deformity of the vascular bed is the primary change in Wernicke's disease. The resulting subacute necrosis of the parenchyma with glial proliferation and proliferation of endothelial and adventitial elements of the vascular walls, as well as the hemorrhages, are secondary. This is equally true for the human disease and for experimentally produced Wernicke's disease in the pigeon.

The topographical analogy of these two conditions is as striking as their histological identity. Figure 7 illustrates the lesions in the mammillary bodies in Wernicke's disease due to chronic alcoholism in man, showing irregular dilatation of vessels, varicose deformities and hemorrhages, which contrast vividly with the finer, regular, normal vascular pattern seen in the remainder of the section. Characteristic varicose deformities from a similar case at higher power are illustrated in Figure 8. Figure 9 shows an experimental lesion in the mammillary body of the pigeon, which in all respects is identical with that seen in Wernicke's disease in man, and which contrasts equally vividly with the normal, finer, regular vascular pattern of the surrounding tissue. Typical varicose deformities from another pigeon are seen at higher power in Figure 10.

In Wernicke's disease due to chronic alcoholism in man the nuclear systems most constantly involved are the mammillary body and the paramedian nucleus (Fig. 11), the periaqueductal region, sometimes only the region ventral to the Sylvian aqueduct (Fig. 12) involving particularly the trochlear and oculomotor

nuclei, the region of the abducens nucleus, the pneumogastric nucleus of the vagus (Fig. 13) and the nuclei of the vestibular nerve, all of them usually involved bilaterally. Figure 14 shows the lesion in the triangular nucleus of the vestibular nerve, which contrasts vividly with the normal, slender, regular vascular pattern in the adjacent nucleus prepositus hypoglossi.

The experimental disease in the pigeon attacks the same nuclei selectively. A sagittal cut through the brain close to the midline may disclose several of these lesions in one section (Fig. 15); namely, in the paramedian region (p) of the thalamus and hypothalamus, the mammillary body (m), the vestibular nucleus (VIII), and the nucleus of the vagus (X). A more lateral section from the same brain reveals an additional lesion in the periaqueductal region involving the nuclei of the eye muscles (Fig. 16). The dilatation and varicose deformities of the vascular bed within the lesion (Fig. 16), quite apart from the hemorrhages, contrast vividly with the regular and slender, normal vascular bed within the neighboring normal tissue shown in the upper third and at the right margin of the figure.

The finer histological changes of the vascular walls (angiodegeneration) are identical in Wernicke's disease in man and in the experimental disease of the pigeon. The characteristic changes in aniline blue stained preparations (Mallory, Masson) are irregular tortuosity of the degenerated vascular walls with delamination of the remaining collagenous strands and hyaline degeneration of the intervening lamellae (compare Figs. 17 and 18). In specimens stained with nuclear stains the degenerated larger vessels of fourth order [8] show intensive proliferation of endothelial and adventitial cells (compare Fig. 19, from a case of Wernicke's disease in man, with Fig. 20, from a case of experimental Wernicke's disease in the pigeon). The precapillaries (vessels of second order [8]) show, with the usual stains, degeneration of their walls (decrease of intensity and uniformity of staining, irregular variations in thickness, and zones of hyaline degeneration) and increase in the number of endothelial and adventitial nuclei, usually in groups (compare Fig. 21 with Fig. 22).

The nerve cells within the lesions show the various types and degrees of Nissl's severe ganglion cell disease. With the Nissl stain they show irregular deformity with accumulation of over-

impregnated masses, such as are seen in Figure 23 in Wernicke's disease in man, and in Figure 26 in experimental Wernicke's disease in the pigeon, partial tigrolysis (Fig. 24 in man, Fig. 27a in the pigeon), and granular deterioration of the cytoplasm (Fig. 25 in man, Fig. 27b, c and d in the pigeon). The nuclei are usually pyknotic (Figs. 23, 24 and 27).

There is an increase of glial elements of all types throughout the lesions, in man as well as in the pigeon (Figs. 17, 22, 26 and 27).

Summary

The lesions of Wernicke's hemorrhagic polioencephalitis occurring in man and a disease produced experimentally in vitamin B_1 deficient pigeons fed ample supplies of other vitamins are identical in their topographical distribution and in their morphological and histological characteristics.

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DESCRIPTION OF PLATES

PLATE 19

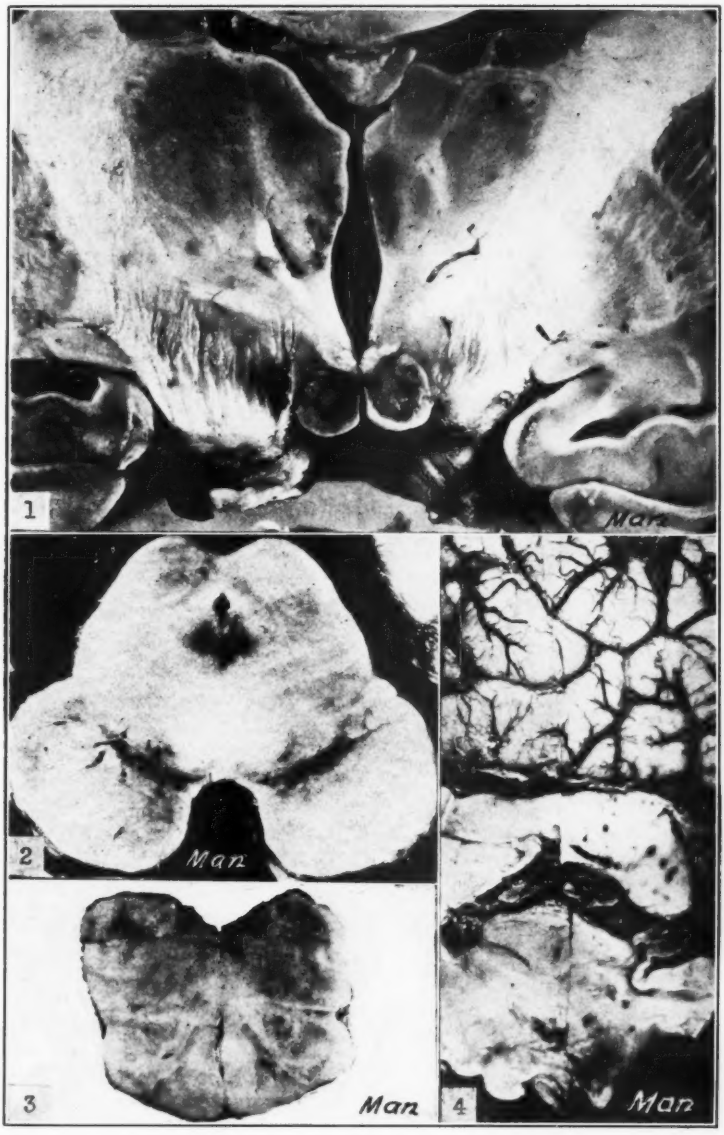
FIGS. 1–3. Fresh, unstained transverse slices through the brain from a case of Wernicke's disease due to chronic alcoholism in a 52 year old male (Case 9).

FIG. 1. Slice through the interbrain. Note the bilaterally symmetrical lesions in the paramedian nuclei of the thalamus and in the mammillary bodies.

FIG. 2. Slice through the midbrain. Note the lesions ventral to the Sylvian aqueduct and in the substantia nigra on each side.

FIG. 3. Slice through the medulla oblongata. Note the lesions in both dorsal nuclei of the vagus, more advanced on the right than on the left.

FIG. 4. From a fresh, unstained, median sagittal slice through the brain from a case of Wernicke's disease with scurvy in a female aged 72 years (Case 7). The corpus callosum, roof and floor of the third ventricle, and the midbrain have been sectioned in the midline, and the medial surface of the right hemisphere and the inner ependymal surface of the third ventricle are exposed. Note the lesions in the paramedian nuclei of the thalamus and the hypothalamus, dorsal and caudal to the mammillary body, and visible through the ependymal covering of the third ventricle.



Alexander Wernicke's Disease

PLATE 20

FIGS. 5 and 6. Wernicke's disease in pigeons produced experimentally by a vitamin B_1 deficiency but with an ample supply of vitamin B_2 (riboflavin extracted from yeast and esterified with phosphoric acid, 0.02 mg. daily) and vitamin C, 13 mg. daily. Fresh, unstained, median sagittal slices through brains. × 8.

FIG. 5. Pigeon 3, Group IV_{2B}. Note the lesions in the paramedian and the paraventricular nuclei of the thalamus and the hypothalamus and in the region of the nuclei of the eye muscles.

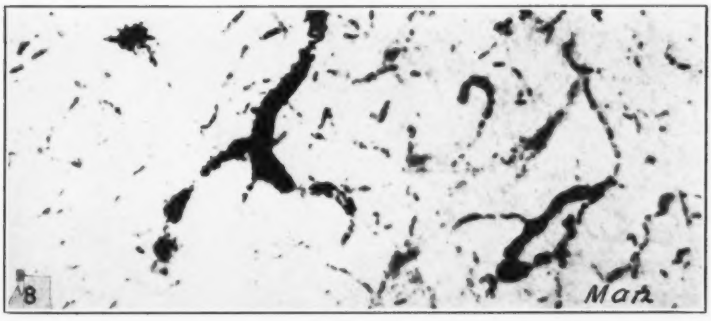
FIG. 6. Pigeon 2, Group IV_{2B}. Note the lesions in the region of the nuclei of the eye muscles and in the paramedian nuclei.

Pigeon
5
Pigeon
6

PLATE 21

FIG. 7. Wernicke's disease in a case of chronic alcoholism in a 28 year old male (Case 10). Transverse section through the ventral part of the interbrain. Note that the lesions are limited to the mammillary bodies. Benzidine stain. × 8.

FIG. 8. From a lesion of Wernicke's disease in the periaqueductal region in a case of chronic alcoholism in a male aged 50 years (Case 8). Note the characteristic varicose deformities of the vessels; a particularly striking one is seen in the right lower quadrant of the figure. Benzidine stain. × 87.

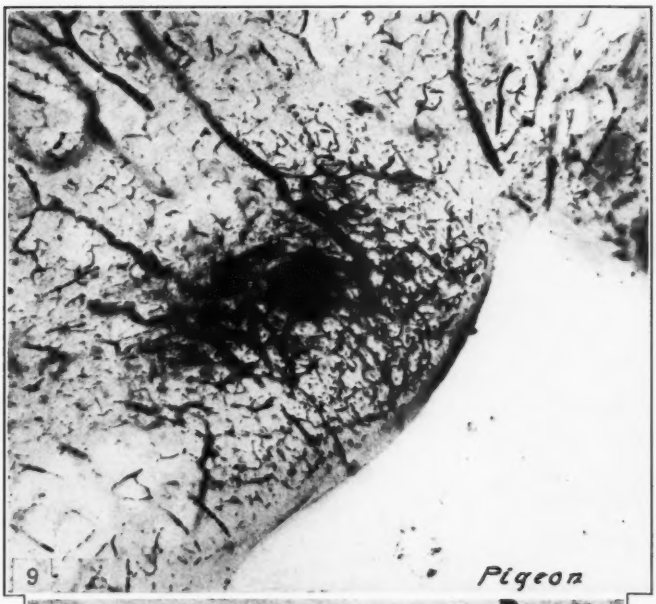


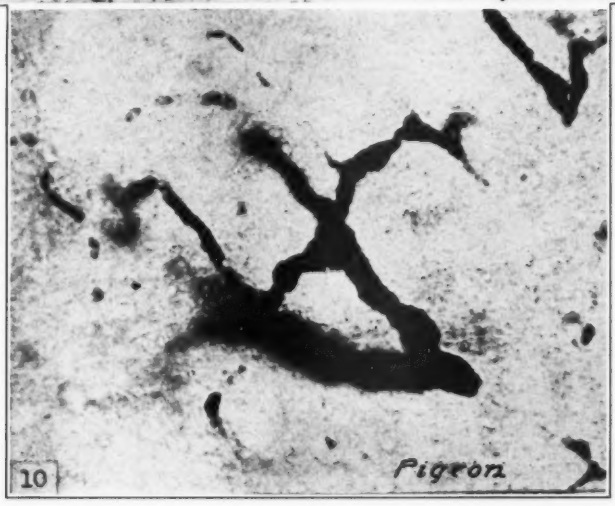
Alexander Wernicke's Disease

PLATE 22

FIG. 9. Wernicke's disease in a vitamin B_1 deficient pigeon which received 0.02 mg. of synthetic riboflavin phosphate daily (Pigeon 1, Group IV_{1A}). Sagittal section through the ventral part of the interbrain. Note that the lesion is limited to the mammillary body. Benzidine stain. × 47.

FIG. 10. From a lesion of Wernicke's disease in the periaqueductal region of a vitamin B_1 deficient pigeon which received 0.02 mg. of riboflavin extracted from yeast and 13 mg. of vitamin C daily (Pigeon 1, Group IV_{4B}). Note the characteristic varicose deformities of the vessels. Benzidine stain. × 280.





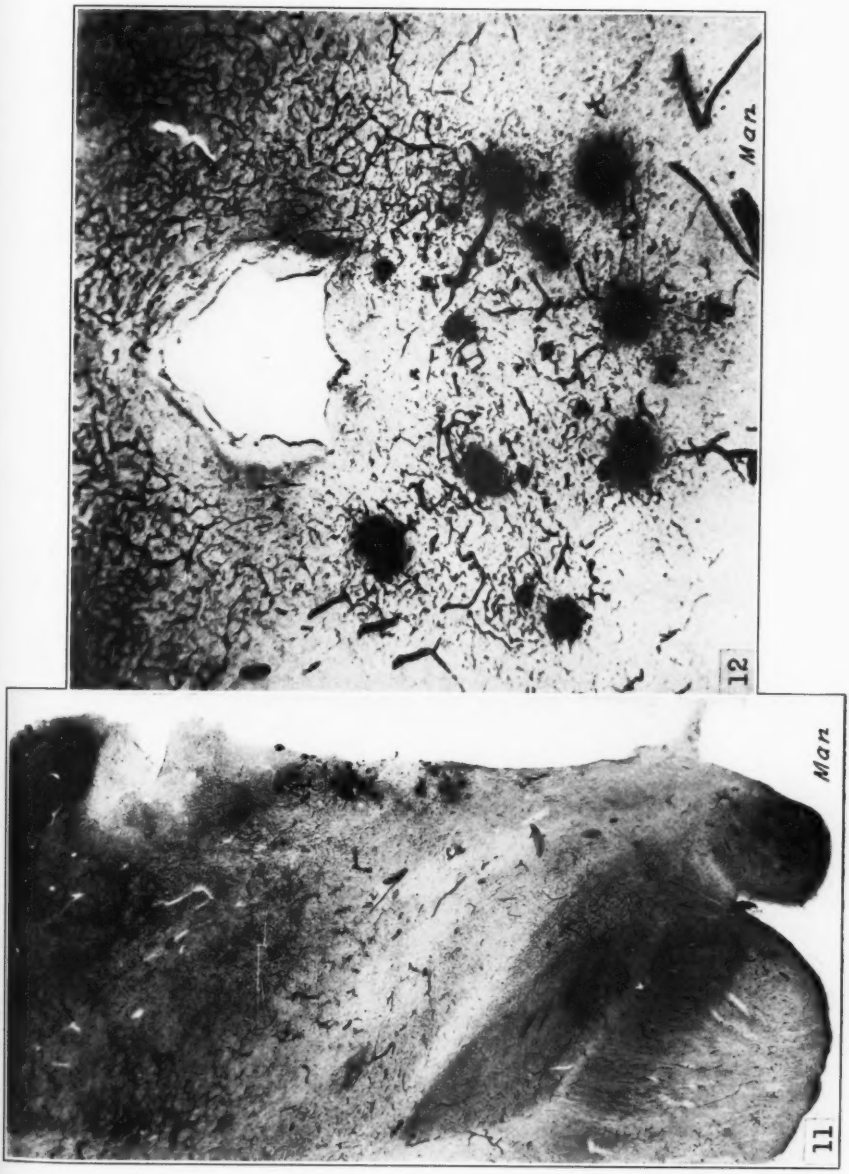
Alexander Wernicke's Disease

PLATE 23

FIGS. 11 and 12. Wernicke's disease in chronic alcoholism in a 52 year old male (Case 9).

FIG. 11. Transverse section through the left interbrain. Note the lesions in the mammillary body and in the paramedian nucleus of the thalamus. Benzidine stain. × 8.

FIG. 12. From a transverse section through the midbrain. Note the lesions ventral to the Sylvian aqueduct. Benzidine stain. × 39.

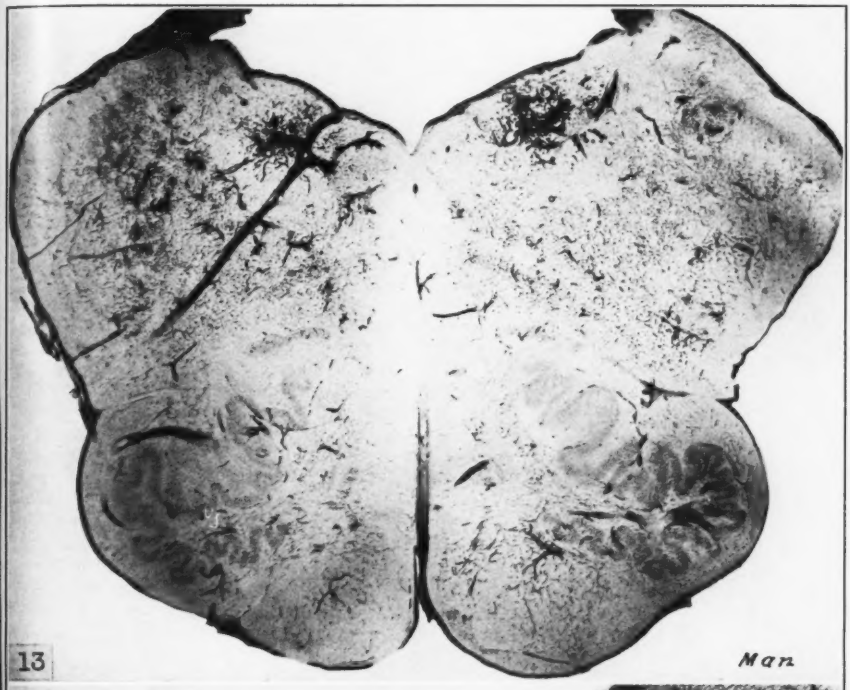


Alexander Wernicke's Disease

PLATE 24

FIG. 13. Wernicke's disease in chronic alcoholism in a male aged 28 years (Case 10). Note the symmetrical lesions in the dorsal nucleus of the vagus, one on each side. Benzidine stain. × 8.

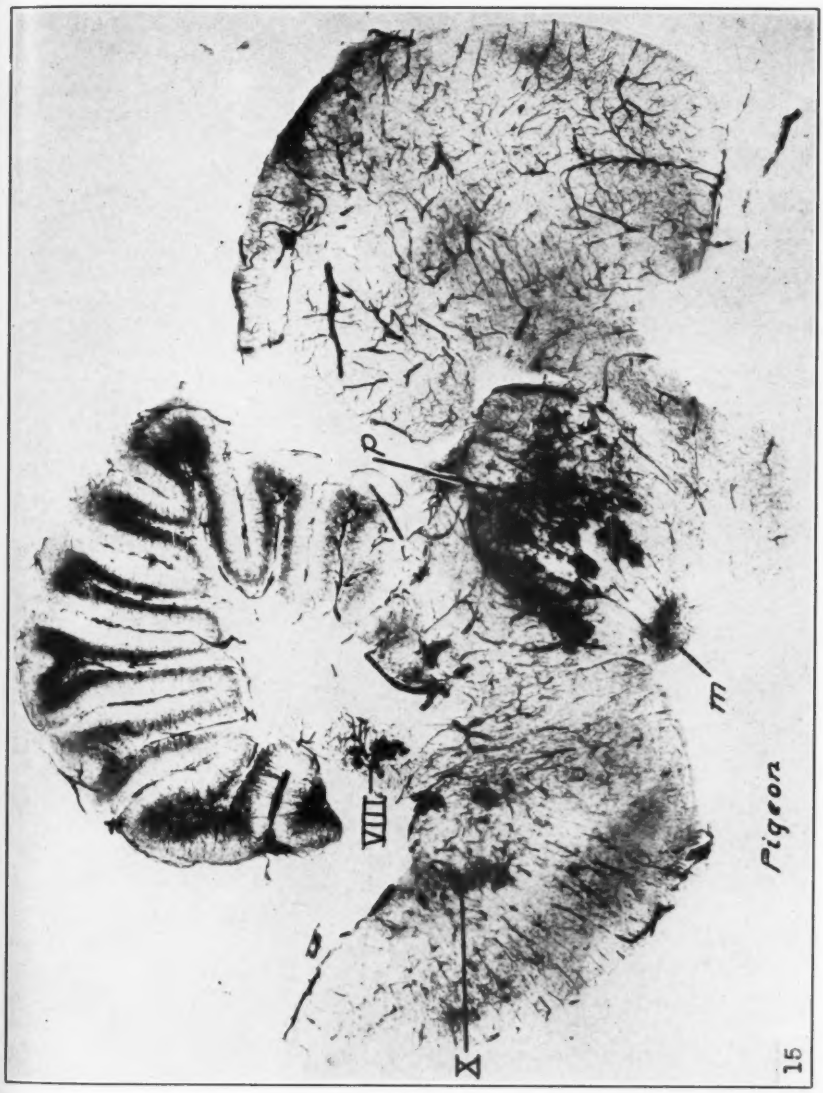
FIG. 14. Wernicke's disease in chronic alcoholism in a male aged 52 years (Case 9). From a section through the floor of the fourth ventricle. Large lesions are present in the triangular nucleus of the vestibular nerve, seen in the upper right quadrant of the figure. Note the normal vascular pattern in the neighboring nucleus prepositus hypoglossi, seen in the lower left part of the figure. Benzidine stain. × 33.


13
Man

14
Man

PLATE 25

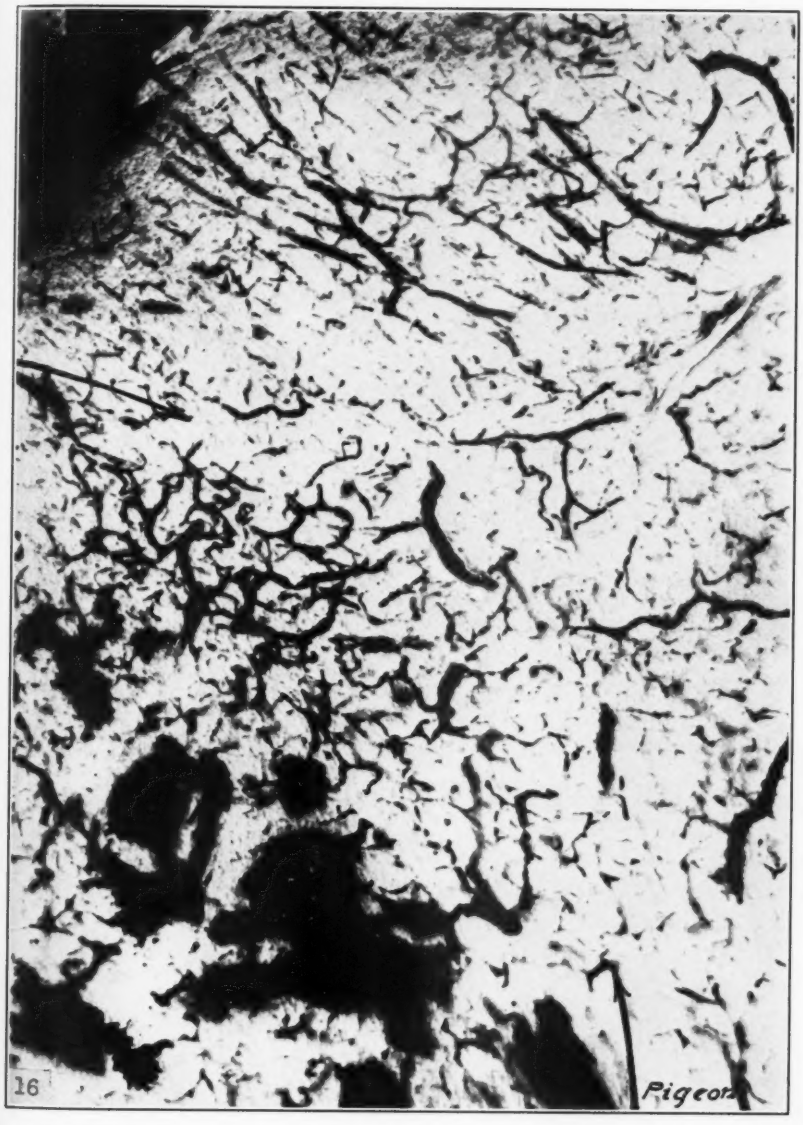
FIG. 15. Experimental Wernicke's disease in the pigeon produced by vitamin B_1 deficiency but ample feeding of synthetic riboflavin phosphate, 0.02 mg. daily (Pigeon 1, Group IV_{1A}). Sagittal section through the brain near the midline. Note the lesions in the paramedian region (p) of the thalamus and hypothalamus, including the paramedian and paraventricular nuclei, mammillary body (m), nucleus Bechterew of the vestibular nerve (VIII), and the dorsal nucleus of the vagus (X). Benzidine stain. × 8.



Alexander Wernicke's Disease

PLATE 26

FIG. 16. Wernicke's disease produced experimentally in the pigeon (Pigeon 1, Group IV_{1A}). Periaqueductal region of the midbrain from a section lateral to that shown in Figure 15 at higher power. A segment of the lesion is shown in the left lower part of the figure, while the adjacent normal tissue is seen in the upper third and at the right margin of the figure. The border between the lesion and the normal tissue can be drawn approximately by a curved line connecting the points of the two arrows which are inserted at the upper left and at the lower margin of the figure. Note the characteristic varicose deformities of the vessels, particularly in the part of the lesion seen to the left of and below the center of the figure. In addition to varicosities, hemorrhages are seen in the left lower corner of the figure. Benzidine stain. × 78.

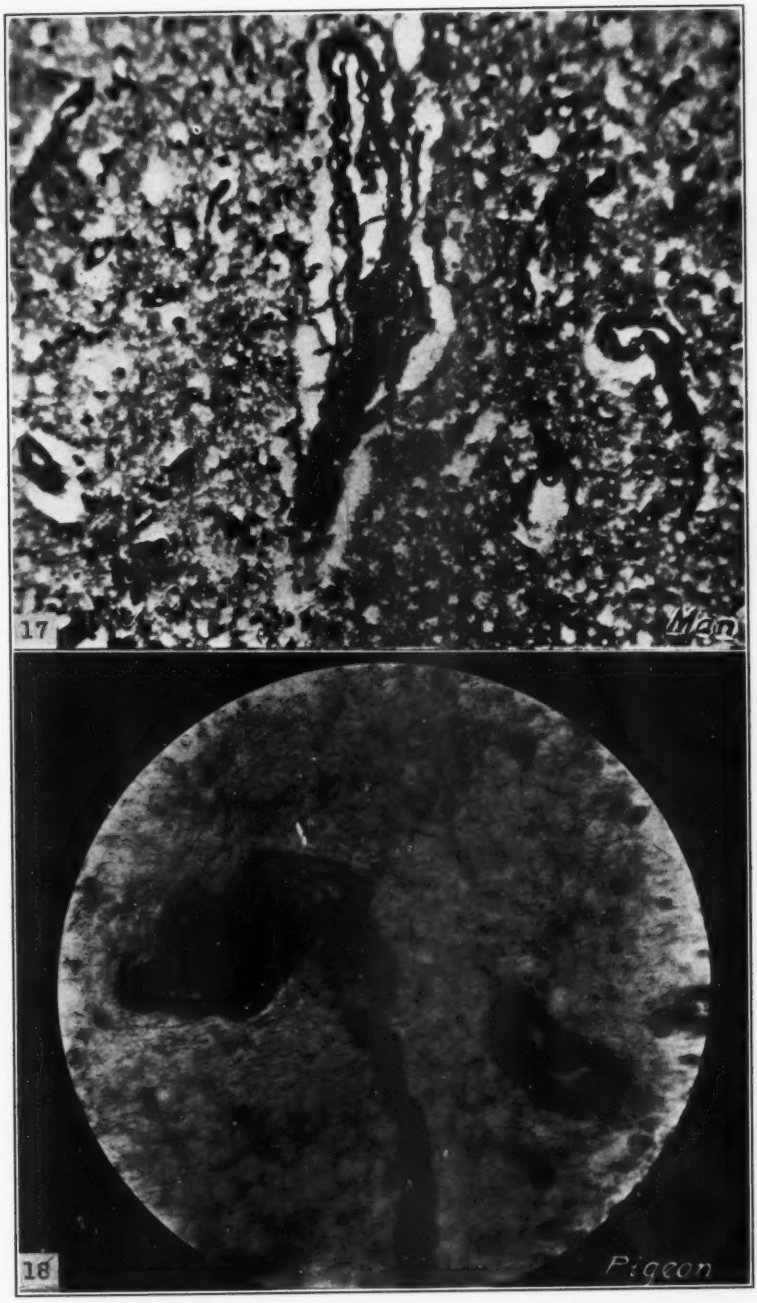


Alexander Wernicke's Disease

PLATE 27

FIG. 17. Angiodegeneration in a lesion of Wernicke's disease in a male aged 50 years, with chronic alcoholism (Case 8). Note the irregular tortuosity of the degenerated vascular walls with delamination of the remaining collagenous strands and hyaline degeneration of the intervening lamellae. Masson's trichrome stain. × 160.

FIG. 18. Angiodegeneration in a lesion of experimental Wernicke's disease in the pigeon (Pigeon 1, Group IV_{1A}, the same bird as illustrated in Figs. 9, 15 and 16). Note the irregular tortuosity of the degenerated vascular walls with delamination of the remaining collagenous strands and hyaline degeneration of the intervening lamellae. Compare with Fig. 17. Masson's trichrome stain. × 335.



Alexander Wernicke's Disease

PLATE 28

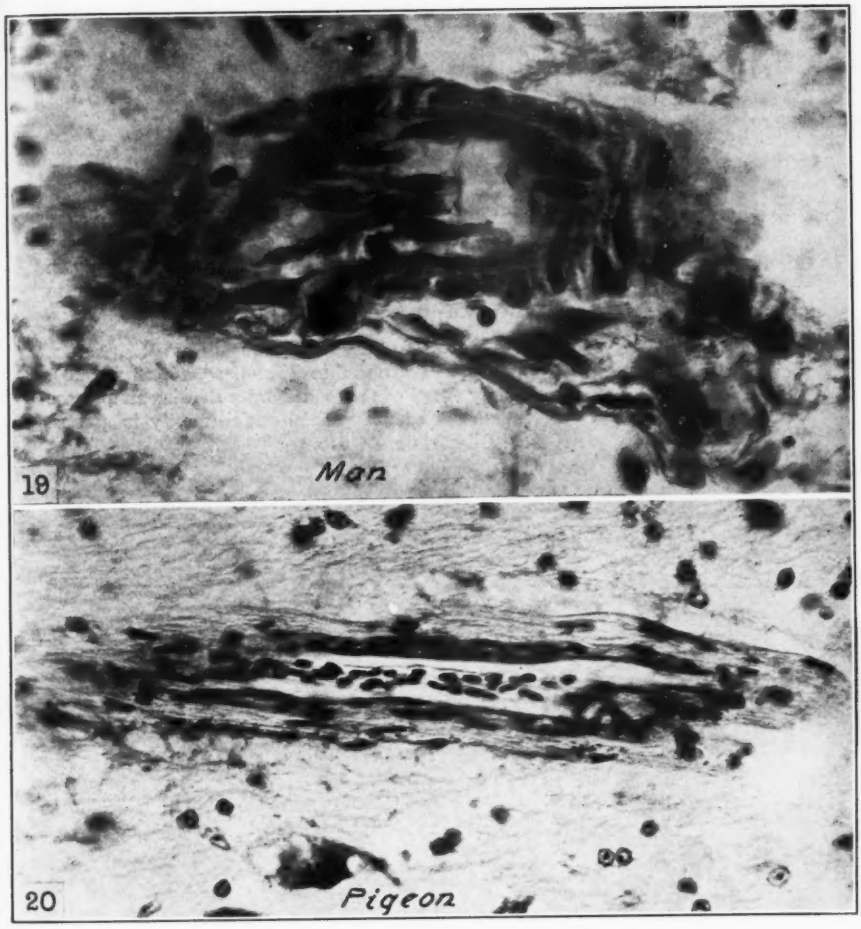
FIG. 19. From a lesion of Wernicke's disease in man (Case 8). A degenerated vessel of the fourth order showing intensive proliferation of endothelial and adventitial cells. Hematoxylin-eosin stain. × 610.

FIG. 20. From a lesion of experimental Wernicke's disease in the pigeon (Pigeon 1, Group IV_{1A}, the same bird as illustrated in Figs. 9, 15, 16 and 18). A degenerated vessel of the fourth order showing intensive proliferation of endothelial and adventitial cells. Compare with Fig. 19. Nissl stain. × 610.

PLATE 28

FIG. 19. From a lesion of Wernicke's disease in man (Case 8). A degenerated vessel of the fourth order showing intensive proliferation of endothelial and adventitial cells. Hematoxylin-eosin stain. × 610.

FIG. 20. From a lesion of experimental Wernicke's disease in the pigeon (Pigeon 1, Group IV_{1A}, the same bird as illustrated in Figs. 9, 15, 16 and 18). A degenerated vessel of the fourth order showing intensive proliferation of endothelial and adventitial cells. Compare with Fig. 19. Nissl stain. × 610.



Alexander Wernicke's Disease

PLATE 29

FIG. 21. A degenerated precapillary vessel of the second order showing an increase in the number of endothelial and adventitial nuclei, mostly in groups, from a lesion of Wernicke's disease due to chronic alcoholism in man (Case 8). Hematoxylin-eosin stain. × 235.

FIG. 22. From a lesion of experimental Wernicke's disease (Pigeon 1, Group IV_{1A}, the same bird as illustrated in Figs. 9, 15, 16, 18 and 20). A degenerated precapillary vessel of the second order showing an increase in the number of endothelial and adventitial nuclei, mostly in groups. Compare with Fig. 21. Nissl stain. × 610.

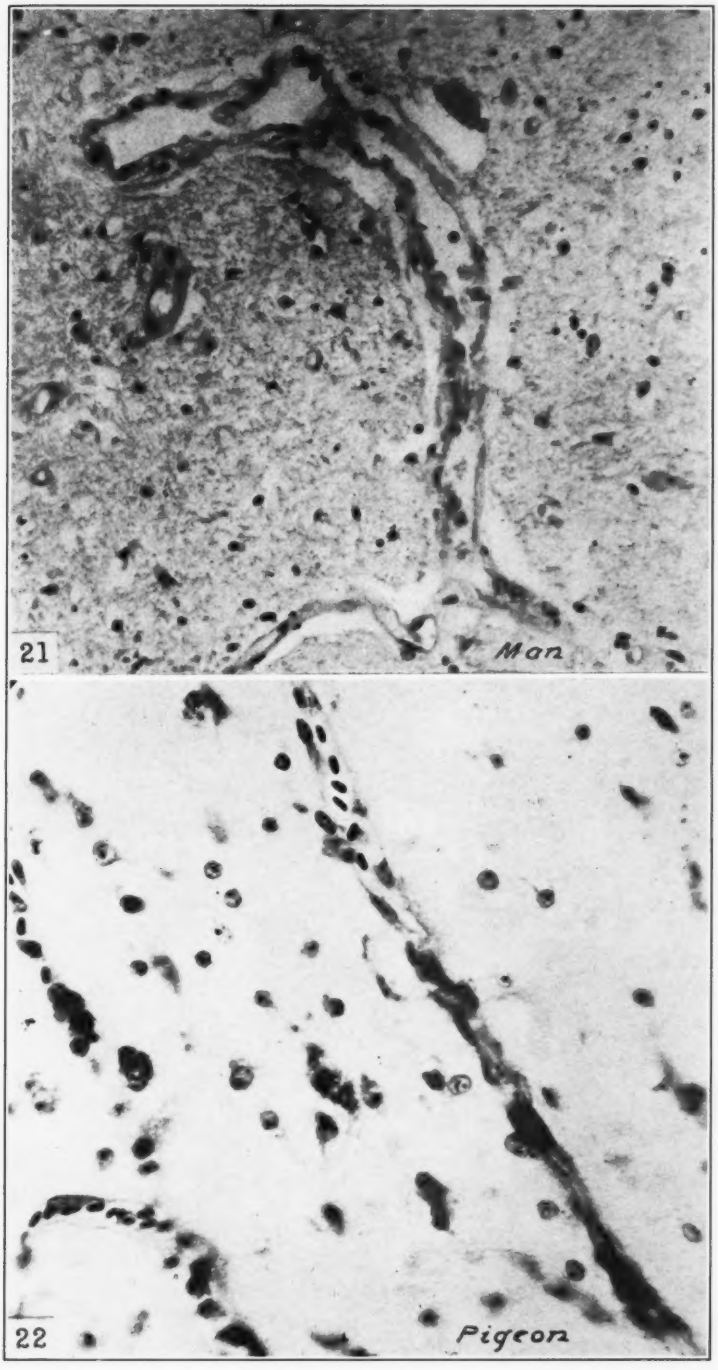

21
Man
22
Pigeon

PLATE 30

FIGS. 23, 24 and 25. Severely diseased nerve cells from a lesion of Wernicke's disease due to chronic alcoholism in man (Case 8). Note the irregular deformity with accumulation of overimpregnated masses (Fig. 23), partial tigrolysis (Fig. 24), and granular deterioration of the cytoplasm (Fig. 25). Nissl stain. × 610.

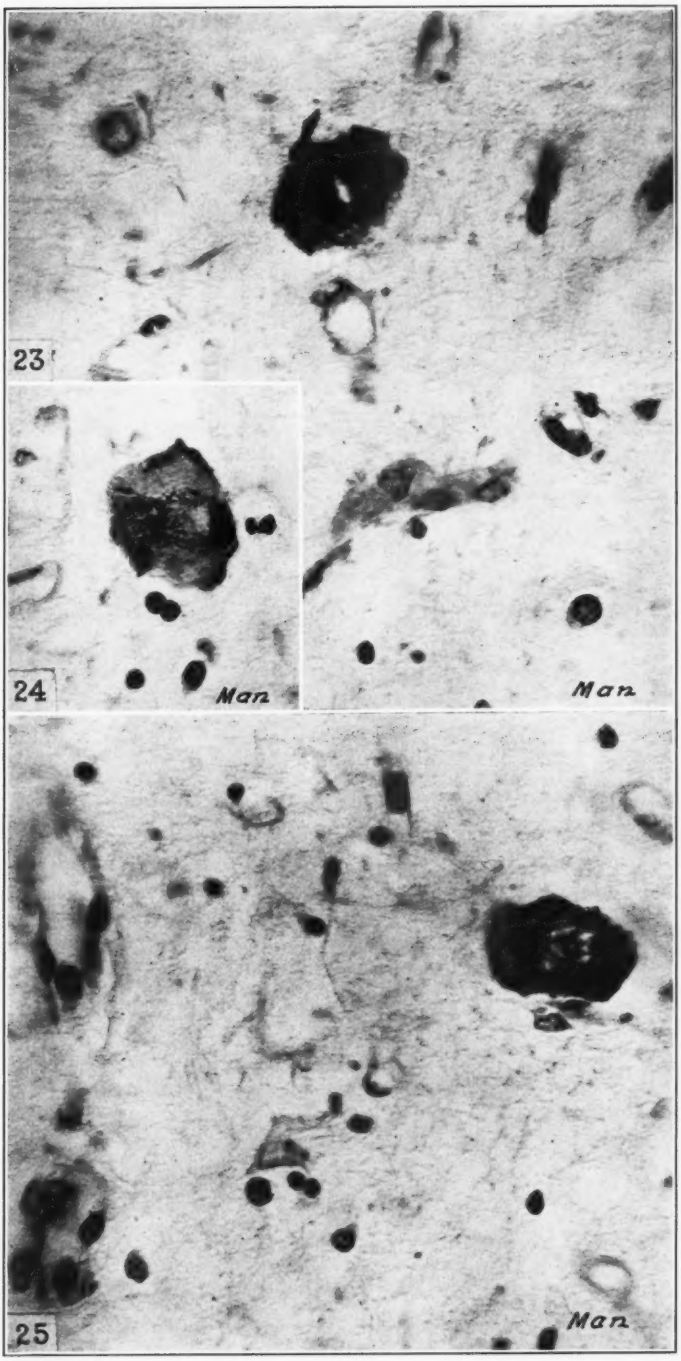
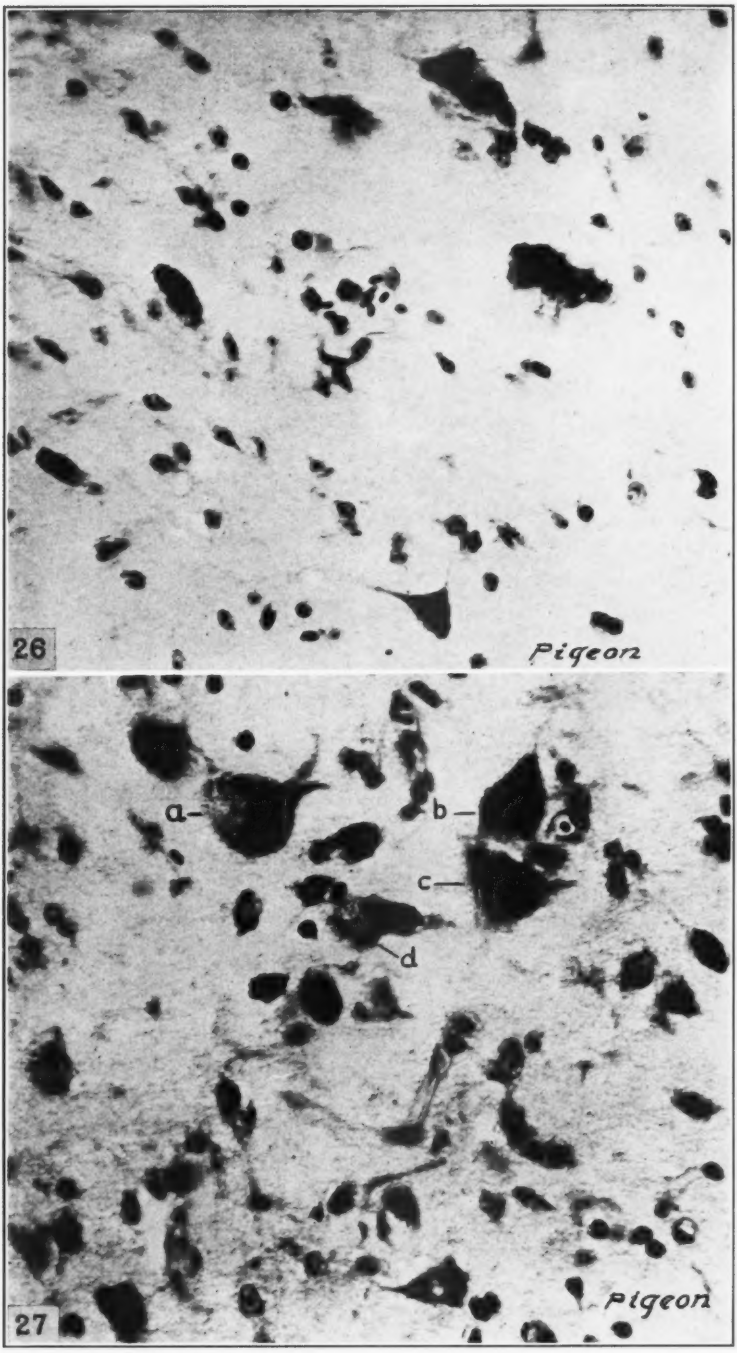

23
Man
24
Man
25
Man

PLATE 31

FIGS. 26 and 27. Severely diseased nerve cells from a lesion of experimental Wernicke's disease in the pigeon (Pigeon 1, Group IV_{1A}, the same bird as illustrated in Figs. 9, 15, 16, 18, 20 and 22). Note the irregular deformity with accumulation of overimpregnated masses (Fig. 26), partial tigrolysis (Fig. 27a), and granular deterioration of the cytoplasm (Fig. 27b, c and d). Nissl stain. × 610.


26
Pigeon
a
b
c
d
27
Pigeon

GROWTH DISORDER OF THE SKULL IN MONGOLISM *

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Ever since human curiosity has made conjectures from the shape of the skull as to the function and activity of the brain hidden beneath its bony envelope, the relation between the brain and the skull has been a subject of great interest to science and philosophy. Phrenology and physiognomy, however, which flourished in the beginning of the last century, did not leave the uncertain ground of speculation until 1858, when Rudolph Virchow [1] made the first attempt to study the development of the skull by histological means. His famous work on cretinism, in which he explained the peculiar appearance of cretins as being due to an early ossification of the base of the skull, proved to be mistaken as far as cretinism is concerned, but his principal ideas about the importance of the synchondrosis spheno-occipitalis and other sutures are generally accepted, and numerous reports have followed his work dealing with the development of the skull.

There are two conditions known which are associated with a marked shortening of the length of the base of the skull — chondrodysplasia and cretinism. My own studies have proved that there is a third entity, mongolism, which also is associated with a failure of the development of the skull in length. Chondrodysplasia and cretinism have attracted much interest and our knowledge of these maladies is fairly well established as far as the histological changes in the cartilage and the bones are concerned. The same is not true of mongolism in spite of the striking appearance of mongoloid children, to whom so much attention has been given since the first description of this malady 75 years ago. There are only a few publications dealing with the development of bone in mongolism, and no histological study of the development of the skull has been published to my knowledge. In 1924 Lauche [2]

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published a paper on mongolism and reviewed the few publications on this subject. He studied four metatarsals and ribs from mongoloid children aged 4, 10, 12 and 18 months, respectively. He mentioned the skull in 1 case but did not discuss the subject.

If we compare histologically the three disorders mentioned above, we may say that in chondrodysplasia growth of the base of the skull is arrested because of lack of development of the cartilage, which is absorbed early and replaced by ossified tissue. In cretinism growth of the bones of the skull is delayed because of lack of transformation of cartilage into bony tissue. The cartilaginous spaces in cretinism are open much longer than normally.

In mongolism the situation is much more complicated. X-ray studies by Siegert [3] in 1910, and also our own x-ray observations, show that the appearance of centers of ossification in the carpal bones takes place early and that ossification comes to an early arrest. In a paper published recently [4] the conclusion was drawn that the cartilaginous bones of the base of the skull show a lack of proliferation and irregularity in ossification. As a result the base of the skull remains proportionately smaller than normal and retains a formation present in the last months of embryonic development. Further development of the normal skull, consisting of growth and molding of the base, is interrupted and pneumatization is lacking. This was concluded because of the x-ray observation that the synchondrosis spheno-occipitalis, which is visible as a small cartilage disc in normal children during the first year of life, and sometimes even later, could not be noted in mongoloid patients after the first few months of life. Therefore, the cartilage may have been replaced by ossification. This assumption, however, is contradictory to the observations of Bean,[5] who reported that the epiphyses of the bones are small and unite late, if at all. As a matter of fact, if the epiphyseal lines of the carpal bones in adult mongoloids are studied by x-ray, a line of almost microscopic thickness crossing transversely through the capita of the carpal bones is sometimes seen. This small line probably represents a remnant of cartilaginous tissue. Lauche observed that the cartilage does not become entirely absorbed and remnants of cartilaginous tissue remain unchanged for a long period of time.

By evaluating these facts, many of which appear contradictory,

the following problem was suggested. Does the cartilage become prematurely absorbed and replaced by ossified tissue, as occurs in chondrodysplasia, or does the cartilage remain unchanged, as in cretinism? What are the histological differences in these conditions? Such an investigation may decide some of the pending questions as to whether the growth disorder in mongolism is associated with an early or a premature ossification of the epiphyseal lines, or with a delay of ossification. If the latter be true, the problem arises as to whether the alterations in mongolism and cretinism are identical, which would suggest a thyroid disorder. If there is a difference in the histological appearance the question will be discussed whether or not this indicates an endocrine disorder and if so of what nature.

Material

Specimens of bones from 8 cases of mongolism were studied. These comprised the synchondrosis spheno-occipitalis from 6 cases, the synchondrosis spheno-ethmoidalis from 2 cases, and vertebrae from 2 cases.

In 1916 Stoccada [6] published a paper on the synchondrosis spheno-occipitalis, its normal development, and the alterations occurring in cretinism. In this paper he described the synchondrosis at the time of birth, during the first decade, the second decade, and after further growth has ceased. My observations on control cases are in accordance with those of Stoccada. Therefore, it may be worth while to record a few points of interest concerning the development of the synchondrosis. The cartilage which lies between the occiput and the sphenoid bone does not form a simple disc at the time of birth but continues to develop toward the posterior clinoid process as a cartilage slide of the same size as the disc, forming the rostral dorsum of the clivus blumenbachii and the posterior clinoid process. The cartilage disc of the synchondrosis averages from 3 to 3.5 mm. in thickness. The cartilage cells form vertical columns in the center and are arranged in horizontal columns toward the occipital bone and the sphenoid body. Growth occurs in two directions. The cartilage disc grows in a vertical direction and the height of the disc increases steadily from about 7 mm. at birth to between 13 to 15 mm. at the age of 17 years. The horizontal cartilage columns proliferate

in the same manner as the epiphyseal lines of the long bones. The thickness of the cartilage disc is about 3 mm. and remains this size for almost the first 20 years of life. At about the age of 20 years the cartilage becomes gradually absorbed and disappears. According to Stoccada the area of proliferation measures between 200 and 300 μ and the area of ossification about 150 μ. The appearance is identical with that seen in the ribs and the epiphyseal lines of the long bones. It may be mentioned that the cartilage proliferation appears slightly more active toward the occiput than toward the sphenoid. After the first year the cartilage frequently shows a tongue-like protrusion into the sphenoid body. Therefore, the synchondrosis appears F shaped. This cartilage tongue was found regularly in our material.

Case 1: A mongoloid male (38–15) aged 7 months and 10 days. The synchondrosis measures 1.5 mm. in thickness and 7 mm. in height (Fig. 1). The cartilage shows small embryonic cells and no preparatory columns are visible. Many small primary marrow cavities are visible without immediate communication with the cartilage areolae. The central medullary cavity is enlarged and touches the cartilage on a broad section by a few, thick, osseous trabeculae. The edge is heavily ossified (see Fig. 3).

By comparison with the measurements of a normal synchondrosis (by Stoccada [6]) the height of the synchondrosis corresponds to the normal, but the width of 1.5 mm., instead of 3 to 4 mm., is much smaller than would be expected. The ossification is obviously abnormal, the growth arrested, the cartilage does not show preparatory involution, and the bony tissue is much more heavily ossified than is seen normally at this age.

Case 2: A mongoloid male (38–25) aged 8 years and 8 months. The synchondrosis is distinctly visible as an F shaped tissue disc between the occipital and the sphenoid bone (Fig. 2). The width of the cartilage is 1.2 mm. A cartilage tongue 1 mm. in length protrudes into the sphenoid bone. The cartilage of the clivus toward the posterior clinoid process is visible and measures 300 μ in thickness. The height of the cartilage disc measures 6 mm. Microscopically small cells are visible in the center of the cartilage running in a vertical direction. Toward the border of the bone on each side the cartilaginous cells are arranged in a horizontal direction. The length of the preparatory zone measures 150 μ.

The capsules contain two or four (no more than six) rather small cells, ovoid in shape. None of these capsules is in direct contact with the medullary cavities; they are closed and entirely embedded in cartilaginous tissue. The border toward the bone is penetrated by numerous, small round cavities containing bone marrow. These cavities are surrounded by cartilage and only a few communicate with the central medullary cavity of the sphenoid body. The rather even cartilage border shows calcification. There are many ossified ridges running in a horizontal direction. Vertical ridges in places separate a second layer of medullary cavities from the central cavity. These cavities are surrounded by heavily ossified tissue and are without communication with other cavities.

Histologically the synchondrosis is much smaller than is seen normally. Growth in the cartilage is arrested. There is heavy ossification of the bone with formation of primary and secondary ridges of bone.

Case 3: A mongoloid female (38–17) aged 9 years and 8 months. The cartilage measures 4 mm. in thickness and 10 mm. in height. It appears to be artificially swollen and its natural width was probably less. The clivus cartilage has entirely disappeared. The cartilaginous synchondrosis fails to show a regular arrangement of the cells and only near the borderline are a few short columns of cartilage cells visible. None of these contains more than two to four small cells. The capsules are without communication with the bone marrow. The cartilage edge is perforated by small marrow cavities which are entirely framed with heavily ossified ridges. The borderline is heavily calcified and thick ridges of bone divide the central marrow cavity connected by transverse ridges which separate smaller marrow cavities (Fig. 4).

The growth in this case appears to be entirely arrested.

Case 4: A mongoloid male (38–32) aged 14 years and 11 months. The cartilage border of the synchondrosis is straight, lacking preparatory cartilage cells. A few, empty, small primary cavities are visible. The edge of the cartilage is heavily ossified. Although this boy was almost 15 years of age, the posterior clinoid process is still entirely cartilaginous. The sphenoid body shows ossified spongiosa, the spaces are filled with bone marrow and the ridges are definitely ossified. No sphenoid sinus has developed.

Histologically this case differs from those described above. The

heavily ossified cartilage border is rather straight. There is only one large central marrow cavity with few ridges of bone and no smaller secondary marrow cavities. The appearance is suggestive of cretinism.

Case 5: A mongoloid male (38–22) aged 20 years and 6 months. The upper part of the synchondrosis is entirely absorbed and replaced by bony marrow. In the lower part is a broad cartilaginous remnant 1.8 mm. in length left which is perforated by a larger medullary cavity. This cavity contains bone marrow. The edges of the cavity are calcified on one side and cartilaginous on the other. The cartilaginous remnant appears to be homogeneous and the small cells do not reveal any direction in arrangement. The edge is even and covered with a thin calcified crust; no signs of further transformation of cartilage into bone are visible. Both tissues are resting. The sphenoid body shows no sinus formation.

Case 6: A mongoloid male (37–8) aged 30 years. In this case no synchondrosis spheno-occipitalis is visible. The sphenoid body is small and shows no sinus formation. In one area a small cartilaginous remnant is visible which was probably left from the cartilage usually separating the anterior and posterior part of the sphenoid body during the first years of life.

Cases 5 and 6 show that the beginning of the resorption of the cartilage spaces occurs within the normal period. The cartilage becomes resorbed without showing signs of activity, the resorption being slow and incomplete.

The Synchondrosis Spheno-Ethmoidalis

The synchondrosis spheno-ethmoidalis, in contrast to the synchondrosis spheno-occipitalis, consists of fibrous tissue. It undergoes ossification according to the process which unites the sutures of the skull and ossifies the membranous bones. Figure 7 shows the synchondrosis spheno-ethmoidalis in a mongoloid boy 9 years and 8 months old. The anterior clinoid processes are rather far apart from the dorsum sellae and no ossification is taking place. Only the edges of the synchondrosis and of the anterior clinoid process are heavily ossified. It is of interest that this synchondrosis forms a fibrous tongue protruding into the sphenoid body. In another specimen from a 15 year old mongoloid child the

synchondrosis spheno-ethmoidalis was also wide apart without signs of ossification. This fact is of interest because Timme [7] described the shape of the sella turcica in mongolism and noticed a peculiar appearance of the anterior part of the sella by x-ray examination. The same observation was later recorded by Tumpeer.[8] Timme interpreted this change to be the result of enlargement of the pituitary gland. Microscopic examination, however, shows that the radiographical appearance is due to the wide open synchondrosis and that the shape of the sella is independent of the size of the pituitary gland in mongolism. Moreover, the appearance of the sella turcica in mongolism corresponds to the x-ray appearance of a full term embryo in which the sella turcica also is separated into two sections.

The Vertebral Column

Figures 5 and 6 show ossification of the cartilage centers of the vertebral bodies. The cartilage of a 2 day old mongoloid child (Fig. 5) shows a normal appearance of the cartilaginous border. There are many preparatory columns present and the ossification appears active. Another specimen from a 2 months old mongoloid infant shows complete degeneration of the cartilage columns. The cartilaginous border is ossified and several primary marrow cavities are recognizable. On the side of the bone tissue there was a huge ridge of bone visible which is parallel in direction with the cartilage border and separates smaller marrow cavities from the central cavity (Fig. 6).

These observations are of interest because in Figure 5, 2 days after birth, the original arrangement of bone and cartilage still appears to be normal. Figure 6, from a mongoloid child 3 months after birth (1 month premature), shows complete degeneration of the cartilage and abnormal bone formation similar to the transverse ridges of bone found in the synchondrosis spheno-occipitalis.

The Ribs

Figure 8 shows the cartilaginous border of a rib from an 8 months old mongoloid child. There are only a few, rather short, preparatory cartilage columns visible. The growth is obviously arrested. However, the histological appearance of the rib cartilage in mongolism is by no means specific. Although there may be a

temporary arrest of growth a comparison of specimens at different ages shows that the ribs grow according to the height of the child. Measurements carried out by Davenport [9] show that the circumference of the thorax in mongoloid children is comparatively normal. The rib, therefore, is not a suitable subject for study of the growth disorder in mongolism and there is no need to dicuss at length the observations reported in the literature.

Discussion

The recorded observations settle definitely the question whether or not mongolism is associated with premature ossification and closure of the epiphyseal lines. Histological studies have proved that such premature ossification does not take place. There is no relation whatsoever to chondrodysplasia. Histologically chondrodysplasia and mongolism differ definitely.

The reports on the mongoloid epiphyseal lines are also of interest in relation to the problem of hyperthyroidism studied by Clark,[10] who considered mongolism as a "fetal hyperthyroidism ceasing at birth," an idea recently reiterated by Meyers.[11] This problem was discussed at length in a paper dealing with the thyroid in mongolism.[12]

In contrast to hypothyroidism, in which ossification is delayed, most authors are of the opinion that hyperthyroidism produces premature differentiation and ossification preceded by a period of stimulation of growth. The obvious retardation of growth in mongolism rules out such an interpretation. Smith and McLean,[13] who recently studied the effect of hyperthyroidism on the growth and the chemical composition of bone in rats, demonstrated that toxic hyperthyroidism may lead eventually to premature cessation or retardation of growth, but this effect is due to toxemia and follows an increase in weight and in substance of bone. In mongolism the bones are small and underweight and no period of increased growth is recognizable. The alterations are associated with the persistence of the skull sutures and the epiphyseal lines of the long bones, which rules out hyperthyroidism.

The question whether or not mongolism is due to a thyroid deficiency and therefore identical with the growth disorder in cretinism is of importance. The growth disorder due to hypothyroidism is well known and is beyond the limits of this paper to

discuss at length. However, a few facts which indicate that the disorder in mongolism is distinctly different from that of cretinism may be summarized as follows:

(1) X-ray observations in cretinism indicate that the appearance of bone centers is delayed and the cartilage spaces remain wide open for a long period of time, even if the normal period of growth has ceased. X-ray observations in mongolism indicate that the appearance of bone centers is normal, but that growth of the bones is minute. Therefore the carpal centers, for instance, remain much smaller than in normal children. In mongolism the cartilage spaces are extremely small, being easily overlooked. A junction of the epiphyseal lines occurs prematurely but some cartilage remnants remain without being absorbed.

(2) From an anatomical point of view the vault in cretinism is always large, the skull being normal or even above normal in circumference while the base of the skull remains small. In mongolism the whole skull is undersized and the circumference, after the first half year of life, is definitely below normal. In mongolism the fontanelles remain open for a rather long period of time. Sutures do not close at the normal time.

(3) From a biological point of view cretinism is definitely associated with high cholesterol values and a low metabolism. In mongolism the cholesterol values are normal and the metabolic rates also.[14]

(4) Although the histological appearance of the cartilage in cretinism and mongolism appears to be somewhat similar at first sight, there are marked differences if observed in more detail. In cretinism the cartilaginous border of bone forms a straight line with few or no primary medullary cavities. The ridge of ossification is rather thin. The preparatory columns show a normal appearance; the cartilage, however, degenerates where lack of thyroid is not corrected by therapy or experimentally produced by complete thyroidectomy. According to Stoccada, this is easily recognizable; the marrow cavities are rare, irregular in shape, and separated from the cartilage by a ridge of bone. The disorder of ossification causes a persistence of cartilage. Siegert [3] and Stoccada [6] interpreted the lack of ossification as being due to a deficiency of the bone marrow which is not able to absorb the cartilage and to stimulate sufficient growth. In mongolism the

histological appearance is usually different, although at times the condition may be confused by a secondary thyroid deficiency (see Case 4). The ossification is active and calcification of the spongiosa is not delayed. There are a number of medullary cavities. The border of the bone is arcade shaped. Lack of proliferation of cartilage is easily recognizable. The cartilage discs are extremely small and proliferation and formation of preparatory columns are arrested.

These observations are in accord with Lauche's studies of metatarsal bones.[2] His Cases 1 and 4, mongoloid children aged 4 and 18 months respectively, showed a complete lack of cartilaginous proliferation. The cartilage was found to be covered with a more or less thick ridge of bony tissue which separated the cartilage from enlarged marrow cavities. In Cases 2 and 3, children 10 and 12 months old respectively, slight traces of cartilaginous proliferation were still recognizable. The bony tissue formed transverse ridges parallel to the cartilage border, which caused the formation of small secondary marrow spaces. Lauche pointed out that in normal children growth goes on during the ossification period, except in the ribs, as long as cartilage is available for ossification. In mongolism this growth is arrested, although enough cartilaginous tissue is still available. In 1902 Kassowitz [15] reported that the marrow spaces were not finger-like processes, but occurred in the form of rounded, knob-like projections into the zone of cell columns, and that consequently the spongy bone did not consist of long tracts of bone, but instead of rounded cavities.

The few studies of the growth of bone in mongolism, some of which seem to be contradictory, are rather well in accord if the same bone structure is concerned. The distal and not the proximal epiphyseal lines are noteworthy. Moreover, one has to bear in mind that growth in mongolism is somewhat irregular and periods of retardation may be followed by periods of more active growth.

The facts gathered above, especially the normal appearance of the centers of ossification but delay of further growth, the small size of the cartilage discs, and the general growth disorder involving not only the cartilaginous epiphyseal lines but the membranous bones of the skull, indicate that the growth disorder is definitely different from that of cretinism. If the arrest of

growth were restricted to the skull, the simplest explanation would be that the lack of development of the brain is the cause of the lack of further development of the skull. Such an explanation is correct for many forms of microcephaly in which the growth of the brain is arrested while the growth of the body continues undisturbed. Although it is true that many microcephalic patients remain dwarfs and that general endocrine deficiency is frequently associated with microcephaly, the arrest of growth of the brain and of the skull precedes the arrest of the growth of the body for many years. Microcephalic patients show a striking disproportion between circumference of the skull and the rest of the body, and observations prove that these patients grow at a normal rate during the first years of life while the skull remains undersized. Not until several years of life have passed does the growth disorder of the body become apparent in microcephaly. The fact, however, that in later life microcephaly is frequently associated with a general endocrine disorder indicates an influence on the part of the brain on the endocrine glands. In mongolism measurements and studies of the brain development indicate that during the first half year of life the weight of the brain corresponds to normal, while at the same time a general growth disorder of the skull and the long bones is recognizable. The arrest of differentiation and the absence of growth stimulation apparently parallel the arrest of further development of the brain but do not depend on it. Moreover, the alterations of the vertebrae and of the long bones are independent of the brain and develop at the same time arrest of growth of the skull occurs.

Comment

By evaluating all factors at hand there is a strong indication that the growth disorder in mongolism is due to the absence of agents which induce differentiation and growth. It is generally assumed that these factors are related in some way to the pituitary gland. Lauche expressed the opinion that the growth disorder in mongolism is the reverse of acromegaly. Studies of the pituitary gland led me also to the conclusion that an insufficiency of the anterior lobe is responsible for the postnatal growth disorder in mongolism.

Erdheim,[16] who studied the growth disorder of the cartilage in

acromegaly, emphasized that in this condition proliferation of cartilage is renewed if cartilage remnants are available (ribs and long bones of young adults). If the cartilage in the epiphyseal lines has disappeared further proliferation is impossible, but the cartilage of the joints shows activity which eventually leads to degeneration. Erdheim is of the opinion that a renewal of cartilage activity is one of the main factors in the acromegalic disorder. On the other hand, hypofunction of the pituitary gland inhibits enchondral ossification and the epiphyseal lines remain open. Erdheim [17] earlier had stressed the fact that the Paltauf dwarf is astonishingly small because the proliferation of cartilage in the epiphyseal lines is completely arrested or markedly diminished through hypofunction of the pituitary. The epiphyseal lines remain open and enchondral ossification is completely or almost completely arrested. The assumption expressed by earlier writers that the cause of dwarfism is a premature ossification of the epiphyseal lines is therefore incorrect, according to Erdheim.

Recent experiments, however, may modify the conclusions drawn by Erdheim. We have to bear in mind that in animals, in whom the epiphyseal cartilage usually disappears, this cartilage will persist if the animals are castrated (McLean *). It is possible, therefore, that the persistence of cartilage in dwarfs and mongoloids is not due to a direct dysfunction but to a hypoplasia of the gonads which is almost always associated with pituitary dysfunction.

Of even more importance in regard to the relation between growth disorders of bone, especially of the skull and pituitary dysfunction, is the work of Mortimer.[18] He studied the effect of hypophysectomy on young rats and his results may be quoted briefly as follows:

"Comparing the measurements of the hypophysectomized and the control, it appears that the greatest disturbance of growth in the hypophysectomized animal's cranium is in the anteroposterior direction, both cranial height and width showing no failure in growth. Occipito-nasal length is 16.1 per cent less and fronto-occipital length is 11.4 per cent less than the control, but the chief defect is in the nasal bones, which are almost a quarter less long than normal. . . . Even more marked is the resultant defect on

* Personal communication.

the lower incisor and mandible, as shown by 34 per cent retardation in the lower incisor and a 43 per cent in the body of the jaw. . . . Absence of the anterior lobe of the pituitary imposes a handicap on growing bone in general which tends, peculiarly and particularly, to affect the growing snout, owing to the cancellous type of bone of which it is largely composed, the brain-case being structurally of more compact bone, which, while undoubtedly affected by hypophysectomy, is so to a less degree."

"The frontal bone is similarly affected in the areas between the vascular expansions, which suffer to a marked degree. This is particularly true in the anterior expansion which occupies the site more closely homologous to the human frontal sinus. It is not only much shorter anteroposteriorly (two-thirds the length), but is also much less in its vertical measurement, while its outer table, instead of forming the convexity of the fronto-nasal angle is depressed and somewhat concave, and its contained marrow aplastic." "A similar condition is present in the basal bones of the skull which are considerably less 'expanded' than normal, and in length are 20 per cent shorter than in the control. The ethmoid and its cribriform plate, as well as the zygoma are considerably smaller."

Mortimer discussed the question that "a possible explanation suggests itself in that growth of the brain-case as a whole is undoubtedly dependent upon growth of the brain; in the hypophysectomized animal the brain is not so large as in the control and can be accommodated adequately in a brain-case which is 11.4 per cent shorter than normal and whose height and width are normal."

It is obvious that the postnatal growth disorder in mongolism is somewhat similar to the effect of hypophysectomy in rats. The mongoloid deficiency is marked by delay in growth in length of the base of the skull, lack of development of the masticatory apparatus, lack of sinus development and pneumatization of the skull, shortness of the nose with depressed bridge, and fetal proportion between brain case and face.[4, 19, 20]

From these observations it is to be concluded that the postnatal development of the mongoloid child is characterized by a marked deficiency of pituitary action. Since the pituitary insufficiency is less pronounced in later life,[19, 21] and the absence of the growth-stimulating hormones is most marked immediately

after birth, the assumption seems to be justified that at the time of birth the pituitary of the mongoloid individual has not reached the normal stage of development which enables the gland to take over the functions imposed upon it by extra-uterine life.

All these data refer to the postnatal development. Mongolism, however, is present at birth and it is still a subject of controversy as to whether the pituitary has much, if any, influence upon the prenatal period of growth. According to Collip,[22] "in early stages of ontogenetic development the organism must have an extra-hypophyseal source of growth-stimulation substances." Mongolism is, apparently, due to a deficiency of the agents that stimulate differentiation and growth during the prenatal and postnatal stages of development.

Summary and Conclusions

1. A histological study of the synchondrosis spheno-occipitalis, of the synchondrosis spheno-ethmoidalis, and of tissue from the vertebrae in mongolism is presented.

2. It is shown that proliferation of the cartilage is absent or insufficient. The histological differences between mongolism, chondrodysplasia and cretinism are demonstrated and discussed.

3. The growth disorder in mongolism is not restricted to the cartilaginous epiphyseal lines but involves also the membranous bones.

4. An analysis of the observations indicates that the development of the growth disorder known as mongoloid deficiency appears to be dependent on a congenital absence or deficiency of those agents which, either from hypophyseal or extrahypophyseal sources, stimulate differentiation and growth.

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DESCRIPTION OF PLATES

PLATE 32

FIG. 1. Synchondrosis spheno-occipitalis (Syn) in a 7 months old mongoloid boy. Width 1.5 mm., height 7 mm. S = sella turcica (posterior clinoid process removed with hypophysis); O = occipital part of clivus. Gross appearance normal. × 8.

FIG. 2. Synchondrosis spheno-occipitalis (Syn) in an 8 year and 8 months old mongoloid boy. Width 1.2 mm., height 6 mm. The disc is shorter and smaller than normal. S = sella turcica (posterior clinoid process removed with hypophysis); O = occipital part of clivus. Note the cartilage slide forming the dorsum of the sella turcica and the posterior clinoid process. × 8.

FIG. 3. Same as Figure 1. Note the absence of preparatory cartilage columns. Many small primary medullary cavities are seen. The cartilage edge is heavily calcified. Note the large central medullary cavity. × 110.

FIG. 4. Cartilage border of synchondrosis spheno-occipitalis in a 9 year and 8 months old mongoloid girl. Note the absence of cartilaginous preparatory columns. Many primary medullary cavities are present. The edge is heavily ossified. Note the formation of transverse ridges of bone. × 110.

FIG. 5. Vertebra from a 2 day old mongoloid baby (body 40 cm. in length). The cartilage shows normal preparatory cell columns. The bone marrow is active with slight irregularities but still within normal limits. × 110.

FIG. 6. Vertebra from a 3 months old (born 1 month prematurely) mongoloid baby (length 52 cm.). Note the complete arrest of cartilage growth. The bone forms transverse ridges, parallel to the cartilaginous border. × 110.

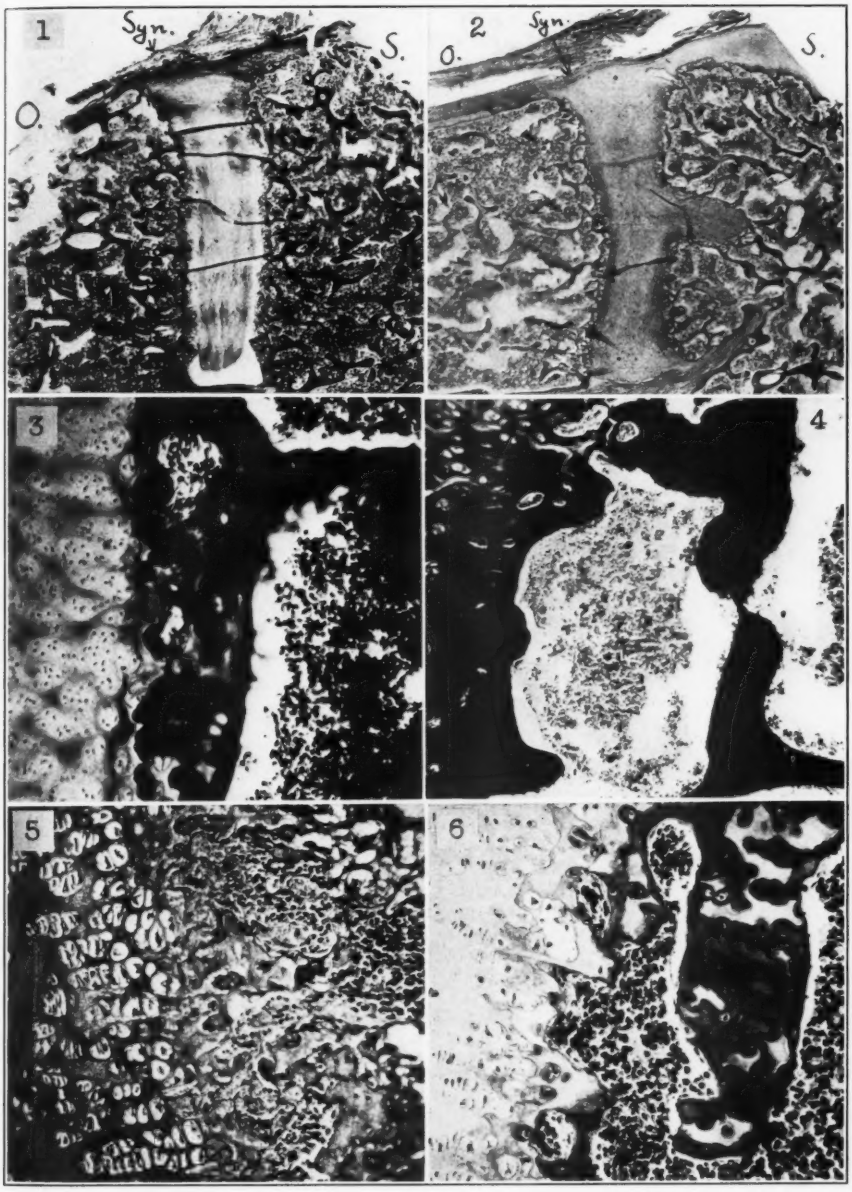
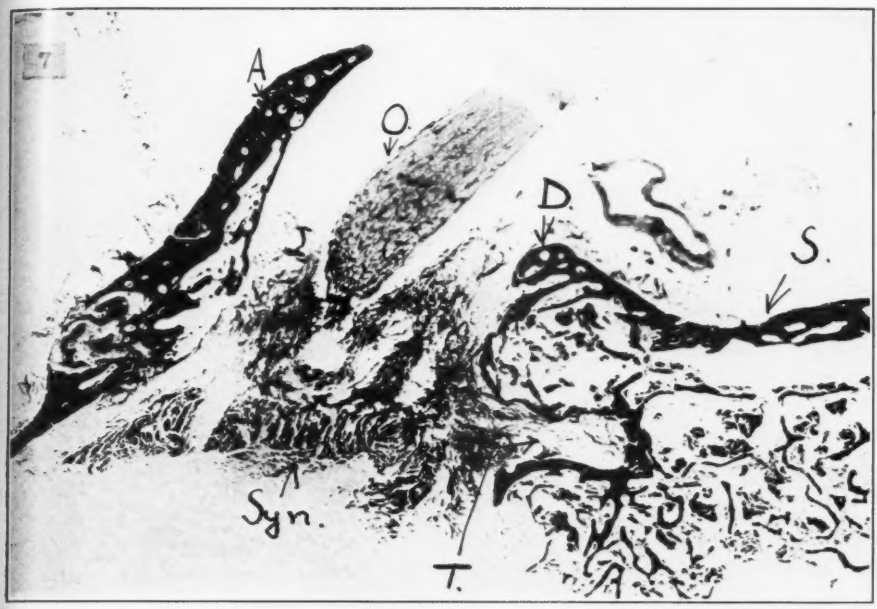

1
Syn.
O.
S.
2
Syn.
O.
S.
3
4
5
6

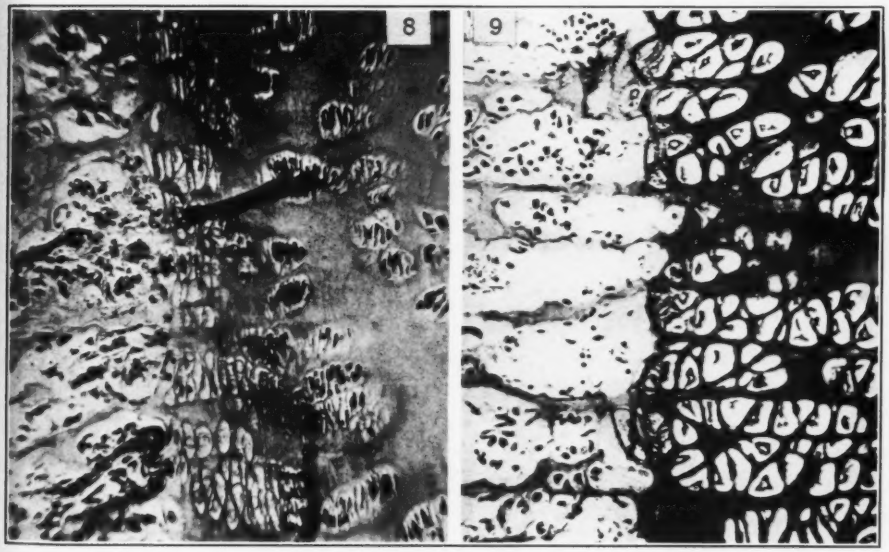
PLATE 33

FIG. 7. Synchondrosis spheno-ethmoidalis from a 9 year and 8 months old mongoloid girl. A = anterior clinoid process; O = optic nerve; D = dorsum sellae; S = sella turcica; Syn. = synchondrosis of fibrous tissue. The synchondrosis is wide open. T = fibrous tongue protruding into the sphenoid body. × 10.

FIG. 8. Cartilage from the rib of an 8 months old mongoloid child. Note the complete arrest of cartilaginous proliferation. The bone appears normal. × 220.

FIG. 9. Cartilage from a normal rib from a control case of the same age. The magnification is identical. Note the large cartilaginous cell columns.





Benda Growth Disorder of Skull in Mongolism

HISTOPATHOLOGICAL CHANGES PRODUCED IN RABBITS BY EXPERIMENTAL INOCULATION WITH HEMOLYTIC STREPTOCOCCI, CERTAIN OF THEIR COMPONENT FACTORS AND SAPONIN *

A Preliminary Report

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In this paper a critical analysis of the histopathological changes produced in rabbits inoculated variously with whole hemolytic streptococci of the Lancefield Group, A, B, C and D, and with certain of their component fractions is reported. It follows logically earlier studies in which a method was described for the preparation and identification of an antigen from each of several types of streptococci following their disintegration by physical means.[1] This antigen has been found capable of removing the type-specific agglutinins from homologous immune serum. Injection of this agglutinogen into rabbits has been found to produce an antiserum which agglutinates (though only to a low titer) the streptococci of corresponding type. The antigen itself is precipitated from solution by antiserums prepared by the injection of agglutinogen of homologous or heterologous type within Group A, and by the injection of whole streptococci of homologous or heterologous type. The agglutinogen has been shown further to be a nucleoprotein which can be separated into serologically active fractions.[2]

On the basis of these preliminary experiments in the disintegration of the streptococcus by sonic vibration it was felt that a study of the tissues of animals inoculated with these several fractions might prove of interest and significance in respect to their utilization as antigenic agents protectively. Accordingly, a series of rabbits was inoculated in various ways and their tissues submitted to

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one of us (L. W. S.) without identifying designation for evaluation of the tissue changes.

Considerably to our surprise and much to our gratification it was possible to sort these tissues out into perfectly definite groups which corresponded in every particular to the source material of inoculation. In the entire series of 65 rabbits only three minor discrepancies occurred, which on careful study of the actual protocol were readily explained. Many of the animals succumbed spontaneously as a result of the inoculation, the time interval varying greatly, dependent on dosage and virulence of the infectious agent, or in some instances of intercurrent or resultant pneumonia. Others were sacrificed at appropriate intervals to complete gaps in the series in respect to time and method of inoculation.

The experimental animals may be divided into 7 major groups with several subclassifications based on the method of preparation of the inoculum or the method of inoculation.

Group 1. Normal Controls: This series consisted of 9 rabbits from healthy laboratory stock which were sacrificed at intervals during the investigation.

Group 2. Rabbits Inoculated with Nucleoprotein Agglutinogen (NPA): This group may be further divided into 2 subclasses: (1) animals injected with filtered material (7 rabbits); and (2) those injected with the unfiltered supernatant fluid which might have contained a few organisms which had escaped disintegration (3 rabbits). This nucleoprotein fraction (formerly designated as labile antigen) is prepared by either of the two methods previously referred to in the paper by Sevag, Lackman and Smolens.[2] The first of these methods is the preliminary disintegration of the bacteria by sonic vibrations of audible frequency; the second, by drying the bacterial mass by the lyophil or cryochem process with subsequent grinding in a low temperature ball mill. The further isolation of the nucleoprotein fraction is carried out by repeated washing, precipitation and final solution in water at a pH of 7.0. It is then usually filtered to remove any remaining fragments of bacterial cells.

Group 3. Rabbits Inoculated with a Rough Strain of the Lancefield Group A Hemolytic Streptococcus: This group consisted of 2 rabbits, 1 of which was immunized by progressive doses of liv-

ing organisms, the other by killed organisms extracted with alcohol and ether.

Group 4. Rabbits Inoculated with Living Cultures of Whole Organisms from a Strain of Mucoid Hemolytic Streptococci of the Lancefield Group A Type: In this group we are obliged to recognize two major subdivisions, each with a further subclass. The first of these represents a series of 15 animals that were immunized with organisms killed either by heat (13 rabbits), or by sonic vibrations (2 rabbits). In both subclasses the animals ultimately received injections of viable organisms after preliminary injection with killed vaccines. In the second major subgroup 7 animals were treated by inoculating a previously induced local subcutaneous blood clot with living organisms in an attempt to reproduce the clinical picture of chronic focal infection. Of these, 5 were successful, resulting in ultimate death of the animal, while in the other 2 the culture apparently did not take and the animals survived.

Group 5. Rabbits Injected with a 9 Per Cent Solution of Saponin, a Known Hemolytic Agent: This group consisted of 4 rabbits used as a control for those treated by the toxic hemolytic fraction of the organism. This particular agent was used because of certain points of similarity, noted in previous experimental work, to the changes associated with the injection of a product believed to contain hemolytic bacterial material.

Group 6. Animals Injected with Reversible Oxidizable Streptococcal Hemolysin: This group consisted of 13 rabbits. It is hoped to further purify this fraction which in its present state is not only *hemolytic* but also *fibrinolytic*. These animals died at intervals of from 3 minutes to 24 hours after injection.

Group 7. Animals Inoculated with Streptococci Other than those of Group A, Including Members of Groups B, C and D: This series consisted of 5 rabbits. Further results in these groups will be reported at a future date. In this series an attempt was made to compare the reaction of these other strains with the typical Lancefield Type A variety.

Pathological Observations

The animal tissues were fixed either in Zenker's fluid or in 10 per cent formalin, and routine sections were prepared from the

heart, lungs, liver, spleen, kidneys and adrenals, and occasionally from other organs including the brain. These were stained routinely by the hematoxylin-eosin method and, in some instances where the live organisms had been used, with bacterial stains to demonstrate the presence or absence of actual organisms in the lesions.

The pathological lesions encountered may be described as occurring in two major forms. The first of these is the condition commonly recognized as parenchymatous degeneration, cloudy swelling or toxic degeneration. This affects the parenchymatous cells of the viscera in varying degree, ranging from minimal granular changes in the cytoplasm of the cells up to actual necrosis. This latter is more frequently encountered involving the liver as a central necrosis, but may at times be found in other organs. Occasionally focal areas of petechial hemorrhage or diapedesis are associated with these severe toxic changes. These changes are ordinarily associated with any toxemia, whether of endogenous or exogenous origin, and are by no means to be considered specific in respect to infections associated with the hemolytic streptococcus.

The second lesion which we feel is definitely characteristic is essentially vascular in nature. We believe it is most probably the result of the action of a circulating toxin, presumably derived from bacteria, regardless of where the actual infection exists. The lesion is identical in its histological appearance with that described by Brody and Smith as occurring in scarlet fever and related streptococcus infections.[3] The process appears to be a progressive one, the earliest manifestations being characterized by a predominance of large mononuclear cells within the capillaries and venules. This is followed or accompanied by a rather striking proliferation of the lining intimal endothelium. In places these cells become almost cuboidal in appearance, and in rare instances actual cell division is visible.

The next step in the development of this lesion is the subintimal infiltration of the vessel wall by various mononuclear cells. This may occur alone but is usually found in conjunction with a marked perivascular cellular infiltration of the interstitial tissues. The lesions in the experimental animals tend to have the same distribution as those observed in the human cases of scarlet fever. The more marked changes are seen involving the capillaries and venules

as an endophlebitis and periphlebitis, but in some of the more extreme cases there may be an actual acute arteritis and periarteritis. The heart, liver and kidneys are regularly involved and it is not at all uncommon to find comparable changes occurring in the spleen, adrenals, lungs, lymph nodes and other abdominal viscera, and even in the central nervous system. In at least 2 cases a severe meningoencephalitis of this order was observed. One point of difference from the scarlatinal cases might be commented on in that the renal involvement is much less marked as compared with that of the heart and the liver.

The third stage is characterized by a marked perivascular, non-suppurative cellular infiltration of the interstitial tissues. The infiltrate is composed chiefly of lymphocytes, occasional plasma cells, and considerable numbers of large mononuclear cells which, from their morphological appearance, apparently represent the usual histiocytes. A further striking feature of the histopathology is the marked tendency toward edema of the interstitial tissues around the vessels. This further suggests a definite primary vascular injury. In the more marked cases, as in those observed in scarlatina, the cellular response which is apparently followed by a considerable fibrous tissue reaction is so striking as to suggest neoplasm. This, of course, is only observed in those cases where the experimental period has been prolonged and has covered a matter of several weeks or even months.

Discussion

While the theory that this reaction is the result of a circulating toxin is perhaps the most attractive interpretation, it must be admitted that the lesions might well result from an allergy which might be likened to a mild Shwartzman phenomenon. The chief reason for introducing this suggestion as a reasonable and plausible explanation for the phenomenon is the fact that in a series of rabbits and mice injected with the commercial Dick scarlet fever toxin these changes could not be demonstrated, nor has any mention of comparable lesions produced by Dick toxin been found in the literature published by other investigators. While the nature of the reaction must, therefore, remain *sub judice*, the characteristic response histopathologically cannot be disregarded and its utilization as a yardstick in studying such experimental material

as that included in this investigation must be accepted both qualitatively and quantitatively.

An attempt to record these changes in the tissues of the animals treated by the various above described methods in the same manner as that utilized in reporting the scarlet fever material has been employed. This is on the basis of a graded scale ranging from 1 to 4 plus, depending on the degree of the various changes already

TABLE I

Summary of Pathological Changes Produced in Rabbits Experimentally Inoculated with Whole Hemolytic Streptococci and their Chemical Fractions

Group	Classification	Number of animals	Parenchymatous or "toxic" degeneration	Vascular lesions	
				Intimal proliferation	Perivascular infiltration
1	Normal controls	9	0	0	0
2	(*a*) NPA (filtered)	7	±	±	0
	(*b*) NPA (non-filtered)	3	1+	±	1.5+
3	Rough strain	2	1.5+	2+	2+
4	(*a*) Whole organisms				
	(a) Heat killed	13	1.5+	2+	2+
	(b) Sonically killed	2	1+	2+	2+
	(*b*) Infected blood clot				
	(a) Died	5	2.2+	2.3+	3.1+
	(b) Survived	2	±	±	±
5	Saponin, 9%	4	3+	0	0.5+
6	Hemolytic fraction	13	2+	2+	3+
7	Group B	1	0.5+	0.5+	1+
	Group C	1	1+	1+	1+
	Group D	3	2+	1+	1.5+

described, which could be quantitatively observed. This is best brought out perhaps by the graphic representation in Table I.

The results of histological study of these experimentally inoculated rabbits may be summarized briefly as follows:

1. No changes, either of toxic parenchymatous or vascular nature were demonstrated in any of the normal control rabbits — Group 1 of the experimental material.

2. At the most, only minimal changes, and these chiefly of a toxic parenchymatous nature as a very minor cloudy swelling, could be found in the rabbits inoculated with the nucleoprotein agglutinogen fraction (the so-called labile antigen of Group 2).

3. The changes in the 2 rabbits immunized by the rough strain streptococcus showed moderate toxic degenerative lesions of the viscera and vascular lesions designated as 2 plus. These changes were perhaps more marked in the rabbit receiving the alcohol-ether extract than in that treated by whole living organisms.

4. In the animals of Group 4, which received the full immunizing course of treatment with killed and subsequently living streptococci, the lesions were surprisingly uniform. They consisted of mild visceral lesions of about a Grade I intensity and of vascular lesions averaging 2 plus in degree. Considerable individual variation was noted from rabbit to rabbit, with the gradation ranging from 1 plus to as much as 4 plus. No appreciable differences were observed in the 2 animals inoculated with organisms killed by sonic vibrations.

Of the 5 rabbits in this group, which were inoculated by means of a subcutaneous infected blood clot, the changes on the whole were definitely more severe than in the preceding group. Toxic manifestations such as hepatosis, nephrosis and myocardosis averaged at least 2 plus, while the vascular lesions tended to show an average figure of 3 plus.

5. In Group 5, the control animals injected with 9 per cent saponin, all showed striking toxic degenerative changes, particularly affecting the liver and evidenced by an acute central necrosis with hemorrhage. Some vascular damage was apparent which was indistinguishable in character from that found in the preceding whole streptococcic group, but it was minimal in amount.

6. In Group 6 the changes observed in the animals inoculated with the hemolytic fraction were primarily vascular in nature, although the toxic degenerative changes, particularly in the liver and kidneys, were also striking.

7. In Group 7, which included the animals inoculated with organisms from Groups B, C and D, the changes observed in the tissues were comparable in kind to those produced by whole streptococci of Group A. They varied considerably in degree but the number of animals was much too small to draw any final conclusions. Suggestively, however, there is a progressive increase in intensity of both toxic and vascular changes, increasing alphabetically, Group B showing the least marked and Group D the most marked changes.

Summary and Conclusions

In summary, a series of animals inoculated with whole streptococci and their various component parts, as well as with saponin as a hemolytic agent for control, suggests: (1) that the lytic fractions of the streptococci are toxic and are responsible for the rather generalized and extensive vascular lesions which occur throughout the viscera of such inoculated animals; and (2) that the nucleoprotein agglutinogen or labile antigen fraction is essentially non-toxic in character, producing no changes of either a toxic or a vascular nature of any significant degree. From this material the hypothesis suggests itself that the nucleoprotein fraction might prove to be of value as an immunizing agent against experimental infection in rabbits, thus avoiding the harmful effects of the toxic lytic fraction present in the whole organism.

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DESCRIPTION OF PLATE

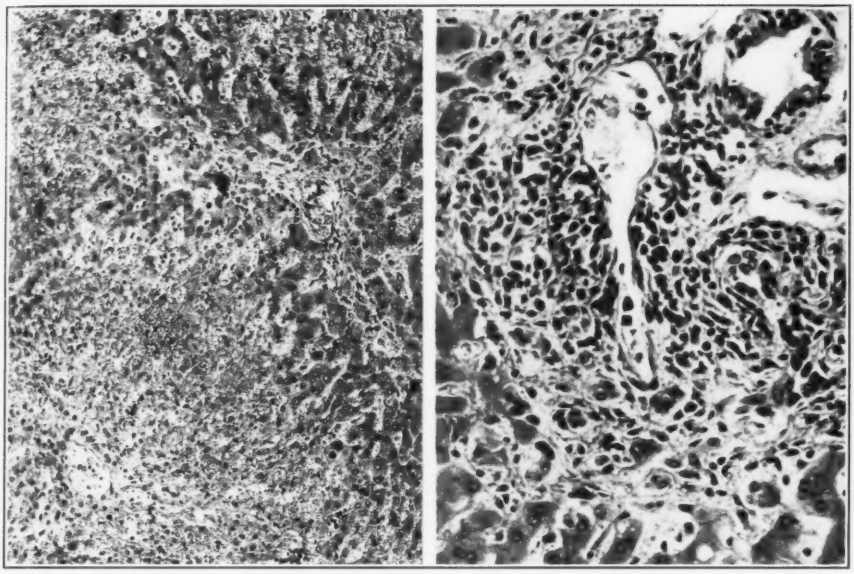
Plate 34

Fig. 1. Section of liver from a rabbit injected with 9 per cent saponin (Group 5) showing marked central necrosis and toxic degenerative changes but no significant vascular lesions.

Fig. 2. Liver from a rabbit inoculated with killed streptococci (Group 3). Note the endothelial lining cell proliferation and the subendothelial and perivascular infiltration by monocytic cells, also the absence of parenchymatous cell injury.

Fig. 3. Section of heart from a rabbit inoculated with a killed culture (Group 4) showing vascular lesions comparable to those seen in the preceding specimen of liver. Note particularly the interstitial edema and the perivascular cellular reaction.

Fig. 4. Section of brain from the same rabbit as shown in Figure 3. Note the presence of an encephalitis resulting from vascular changes similar to those observed in other organs.



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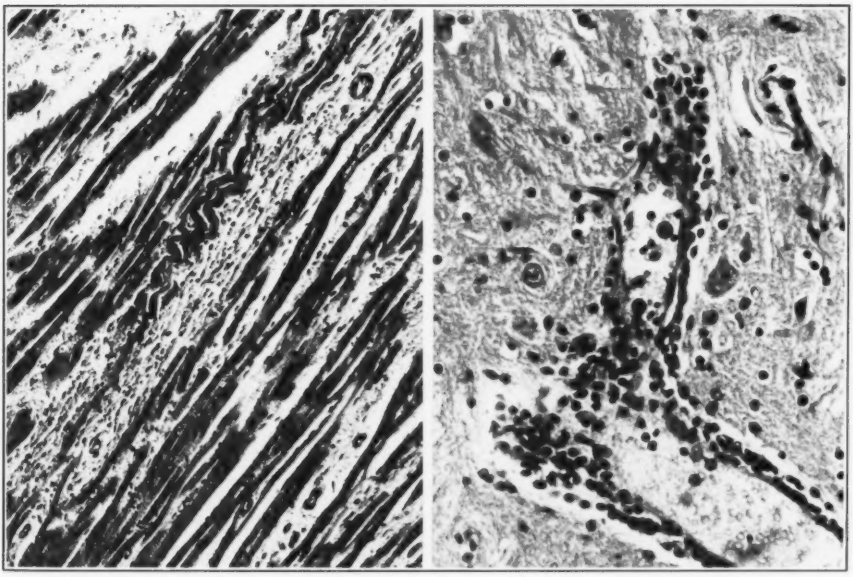

3 4

Smith, Morgan and Mudd Histopathological Changes Produced in Rabbits

EXPERIMENTAL BOTRYOMYCOSIS *

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Two cases of botryomycotic granuloma of the intestinal wall produced by fish bones were recently reported.[1] It was concluded that the formation of botryomycotic granules is brought about by a foreign body reaction associated with a staphylococcus infection. Subsequently an attempt was made to produce this lesion experimentally and lesions were obtained which we believe to be essentially identical with those seen in human cases. It was the purpose of these experiments to determine the conditions under which such granules are formed and to study their histogenesis.

Most authors have accepted Magrou's theory,[2] which assumes that botryomycosis is caused by an infection with a limited, relatively small number of staphylococci. Magrou based his opinion on two facts: (1) staphylococci can always be isolated from spontaneous botryomycosis; and (2) he was able to reproduce this lesion experimentally by inoculation with various strains of staphylococci. Although the conditions under which spontaneous botryomycosis develops suggest that the effect of foreign bodies should be considered an important factor in its pathogenesis, Magrou reported that he obtained characteristic granules without a foreign body reaction. As far as can be ascertained, his experiments have not been repeated, nor are we aware of any other successful attempts by different methods.

EXPERIMENTAL

In order to determine whether or not foreign bodies play an essential rôle in the formation of this lesion, we carried out four different types of experiments, using the same strain of staphylococci in all. We first repeated Magrou's experiments, following his technic as closely as possible. Horse hairs dipped into a suspension of staphylococci (a 24 hour culture on an agar-agar plate

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suspended in 3 cc. of salt solution) were pulled through the testes of 6 guinea pigs. The results were negative, whether the horse hairs were left in the testes or withdrawn immediately. Although a different strain of staphylococcus or a greater number of organisms might possibly have been more successful, we believe our negative results to be significant in comparison with those obtained by other methods.

Staphylococci were next injected into areas of foreign body granulation tissue produced in rats by injecting talcum powder intracutaneously and subcutaneously. Approximately 0.2 cc. of a suspension of staphylococci in various concentrations was injected into some of the nodules produced after about 8 to 10 days. The control nodules remained unchanged and were freely movable under the skin during the following period of 2 weeks. The infected nodules, particularly those injected with suspensions of higher bacterial concentration, became adherent to the skin, superficial necrosis developed, and ulceration remained for about 1 week before healing took place. The inflammation gradually subsided after approximately 3 weeks. Sections taken at various intervals revealed foreign body granulation tissue with superimposed, acute inflammatory infiltration, abscess formation and superficial ulceration. Histologically the process was non-specific in nature, no botryomycotic granules being identified. Control injections with staphylococci produced small subcutaneous abscesses without ulceration through the epidermis.

Fish bones which had been immersed for 2 days in broth cultures of staphylococci were then implanted into the peritoneal cavities of 6 rabbits.* Control rabbits, in which fish bones containing living staphylococci were implanted in the bone marrow, showed that the staphylococci multiplied.

Unwashed fish bones were next implanted; some were superficially cleaned with salt solution and others were dipped in 95 per cent alcohol for 30 seconds. Embedded in a pouch of peritoneum, the fish bones were fixed against the outer surface of the intestinal loops. After intervals of from 1 week to 2 months the rabbits were killed. All fish bones were found at the site of implantation with a minimum amount of foreign body granulation tissue without noticeable acute inflammatory infiltration. Except for local ad-

* Two fish bones in each rabbit.

hesions, the peritoneum was smooth, moist and glistening throughout. Botryomycotic granules could not be demonstrated.

Finally, sterile fish bones, and those contaminated with staphylococci, were pierced through the wall of the intestines of 7 rabbits. The blunt end of the bone was fixed against the peritoneum with the pointed end freely movable in the lumen. The site of penetration was tightly covered with a peritoneal fold. The rabbits were killed at intervals of from 2 to 62 days. In some animals little or no change had taken place, but in others tumor-like granulation tissue was present in the intestinal wall which varied in size from a few millimeters to 2 cm. in diameter. The granulomas projected several millimeters to 1.5 cm. above the peritoneal surface and were frequently adherent to adjacent intestinal loops. All were situated opposite the freely movable, pointed ends of the fish bones, according to the length of the latter, about 2 to 2.5 cm. from the site of penetration, which was invariably covered with smooth peritoneum and showed little or no grossly visible inflammatory reaction.

In these experiments we found that dogs are unsuitable because the part of the fish bone inside the intestinal lumen is completely digested within a few days in every instance, only a small stump remaining in the wall, its blunt end smoothly covered by peritoneum. In rabbits the granulomas developed in the small intestine, as well as in the large intestine, the sterility of the fish bone being apparently of no importance. Grossly visible infiltrations of the wall were obtained in about two-thirds of the rabbits and in several botryomycotic-like granules were observed histologically.

Comment

From our observations we conclude that the sequence of events is as follows: At first the continuity of the mucous membrane is interrupted in several small areas opposite the pointed end of the fish bone. The floor of these resulting ulcers is covered with débris, fibrin and bacteria. The submucosa is markedly edematous and reveals a slight infiltration with small round cells and numerous eosinophils. No bacteria are found in the submucosa, but organisms are present in the surface areas. Even in the later phases the erosions of the mucous membrane remain superficial, being permanently maintained by continuous mechanical irrita-

tion. That elements of the intestinal contents enter the lymph spaces of the submucosa and travel in lymph channels through the entire wall toward the serosa is shown by the fact that foreign particles, such as plant cells, surrounded by polymorphonuclear leukocytes, are demonstrable in the lumens of dilated lymph spaces, the endothelium and surrounding tissue of which appears normal. A few, scattered Gram-positive cocci accompany the foreign particles on their way through the intestinal wall. After 1 to 3 weeks these elements arrive and accumulate in the serosa where an extensive and rather characteristic granulation tissue develops, composed of fibroblasts and round cells of various types with diffusely scattered foreign body giant cells. This granulation tissue contains numerous, sharply delineated foci apparently consisting of dense débris which takes a dark blue and mottled red color with the hematoxylin and eosin stain. In some foci fragments of foreign particles are still recognizable. All contain irregularly shaped, purplish bodies floating in the mass of débris. These bodies are homogeneous, with sharp, frequently doubly refractive borders. They are apparently hard in consistence. In the later phases these bodies are frequently seen exterior to the masses of débris, *i.e.* in the midst of fibrous granulation tissue surrounded by a narrow zone of small round cells and polymorphonuclear leukocytes. They frequently, particularly in this latter instance, show easily recognizable club-like excrescences. It is assumed that either the débris is finally absorbed and the indigestible hard bodies remain, or that they are carried away by lymph channels into the adjacent granulation tissue. It can be shown that these bodies are not derived from foreign material but are the products of degenerated connective tissue elements. The granulation tissue at the border of such foci of débris condenses into a hyaline pinkish mass. This, at first, projects with club-like processes into the mass of necrotic material, still in continuity with the adjacent tissue. It then crumbles off and forms the bodies described above.

It is noticeable that bacteria are absent throughout the granulation tissue, and even within the foci of débris, except at their borders at the site of the hyaline-like condensation. Colonies of Gram-positive cocci cover the surface of the club-like projections, accumulating at their tips and being carried away with them. Soon, however, they degenerate and lose their staining qualities.

They can no longer be demonstrated in the bodies in the midst of the débris, nor in those which are found isolated in the granulation tissue.

Conclusions

We believe that the lesions obtained in our experiments are essentially identical with botryomycosis, although this statement warrants critical examination. As pointed out in a previous paper, the criteria of botryomycosis are not satisfactorily established. According to present opinion the typical botryomycotic granules consist of a matrix probably derived from tissue and bacterial débris. The bacteria sometimes described as dead and coalescent (Plaut [3]) are crowded at the periphery of the granules, which are characterized by club-like excrescences and surrounded by a hard, doubly refractive cuticula. However, both the membrane and the club-like excrescences apparently are not always demonstrable.

Many of the granules obtained experimentally have a striking resemblance to those seen in human cases and described in the literature. They have club-like excrescences and a partially doubly refractive border. It is true that a distinct cuticula with a specific staining reaction, such as that described by Berger *et al.*,[4] could not be identified in the granules in our experimental lesions. It is also true that bacteria could not be demonstrated in the late phases of development. In the earlier stages, however, numerous colonies of staphylococci are concentrated on the surfaces of areas of condensed necrotic tissue which forms the granules after completion of their detachment. Although the bacteria lose their staining qualities and apparently die at an earlier phase of development than in the cases of botryomycosis reported in the literature, they obviously play an important rôle as the causative agents in our experimental lesions. It must be emphasized that the granulation tissue does not contain any cocci except at the very site of the developing granules. Hence, in spite of these differences, we feel justified in identifying the bodies we obtained experimentally as botryomycotic granules.

The histogenesis of these granules is similar to that recently reported in 2 human cases.[1] The connective tissue, apparently under the local influence of bacteria, degenerates, condenses and forms the matrix of the granules. As in the human cases, there is

no polymorphonuclear leukocytic exudation in the immediate vicinity of this necrosis. In later phases, however, when the granules come into immediate contact with the surrounding tissue, an inflammatory exudate with polymorphonuclear cells takes place.

Considering the pathogenesis of botryomycosis, we have pointed out that the effect of foreign bodies could not be overlooked as an essential factor. Summarizing the results of our experiments, we can state that botryomycotic-like granules cannot be obtained by a single inoculation of staphylococci whether accompanied or preceded by the introduction of foreign material. Botryomycotic-like granules, however, are found if the granulation tissue is perpetually supplied by foreign material contaminated with staphylococci. In our last experiments ulcerations of the mucous membrane were permanently maintained and foreign particles with a small number of staphylococci continuously traveled through the intestinal wall toward the serosal granulation tissue. All other experiments provided but one inoculation with staphylococci and while the foreign body reaction was still active the tissue was capable of coping with this single infection.

From the study of human cases of botryomycosis it was concluded that the granules are formed because foreign body granulation tissue may possibly have a reaction different from that of normal tissue secondarily invaded by bacteria. In the light of these experiments, however, this statement needs qualification. It is evident that it is not the foreign body which alters the tissue reaction in such a fashion that simultaneous staphylococcic infection leads to the formation of botryomycotic granules. This takes place only if the invasion of bacteria is perpetuated. It is the continuous inoculation with a small number of organisms which causes the granulation tissue to degenerate in this peculiar manner. It cannot be demonstrated that the foreign body has any effect other than that of providing ways and means of perpetuating the infection. It is obvious, however, that the virulence of the staphylococci is thereby altered. The very strain that in single subcutaneous injections produces an abscess is limited in its growth under such conditions and lacks a polymorphonuclear leukocytic reaction. The foreign body granulation tissue does not reduce the activity of staphylococci since inoculation into granulation tissue produced by injection of talcum powder resulted in superimposed

suppuration. It appears, therefore, most likely that the decreased virulence of the staphylococci and the peculiar alteration of the tissue reaction are the direct result of the perpetuated infection with small numbers of bacteria.

This theory seems to us to be in keeping with the circumstances under which spontaneous botryomycosis occurs.

Summary

1. Foreign body granulation tissue was experimentally produced in the intestinal wall of rabbits by implantation of fish bones.

2. If accompanied by a perpetual infection with small numbers of staphylococci, granules are formed which are identified as botryomycotic granules.

3. Botryomycosis, therefore, is believed to be due to a continuous invasion of the tissue with staphylococci.

4. Foreign bodies, such as bones, and so on, provide a way of perpetuating the infection.

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DESCRIPTION OF PLATE

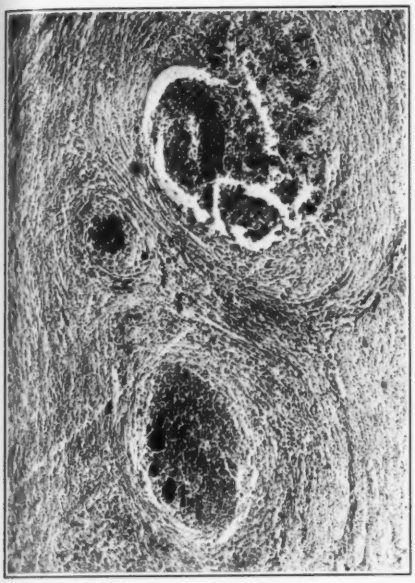
PLATE 35

FIG. 1. Foci of débris within the granulation tissue with deeply stained, condensed bodies.

FIG. 2. Border of a focus of débris. Gradual necrosis and hyaline condensation of surrounding tissue is seen. Hard fragments have crumbled off.

FIG. 3. A broken-off fragment under high power with a partially, doubly refractive border. A Gram stain reveals numerous colonies of Gram-positive cocci at its surface.

FIG. 4. A botryomycotic-like granule with club-like excrescences surrounded by polymorphonuclear leukocytes.



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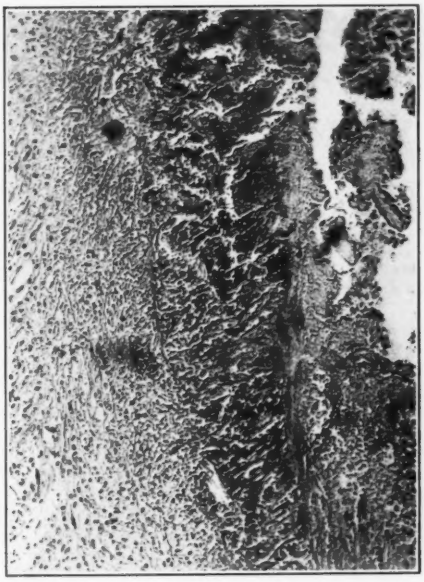

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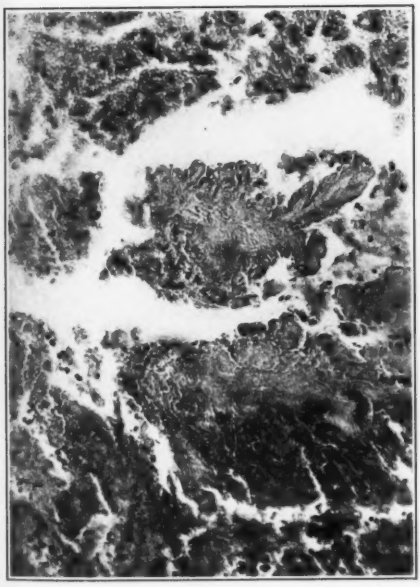

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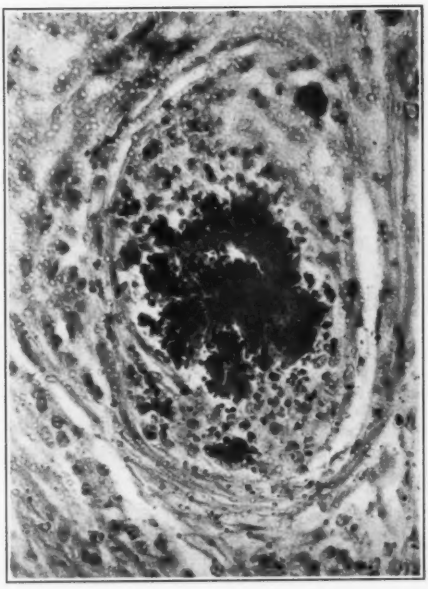

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Kimmelstiel and Easley — Experimental Botryomycosis